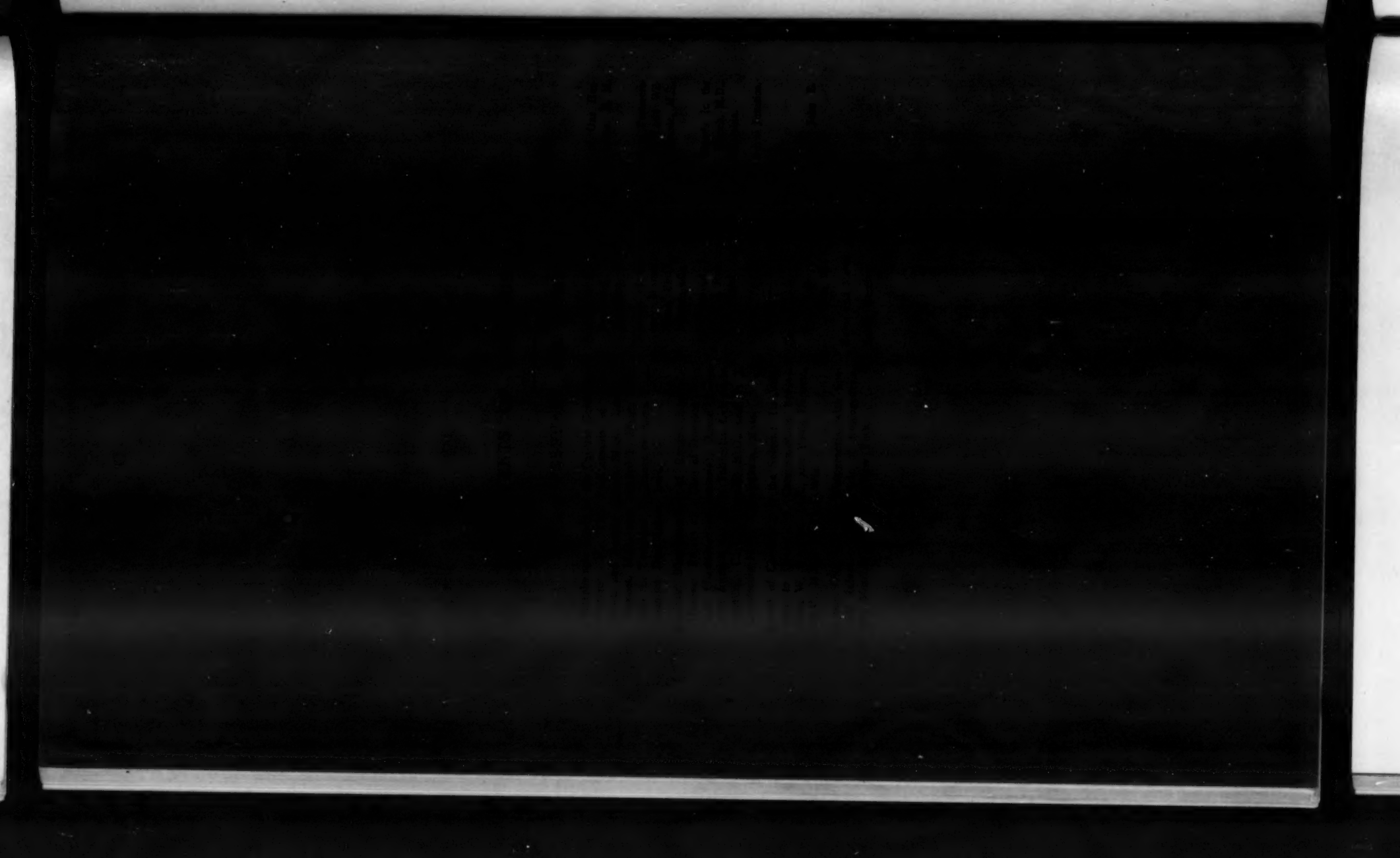

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CHRONIC INFECTION AND ATHEROSCLEROSIS

Some Additional Experimental Data

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THERE is known to be some clinical and experimental evidence which suggests the possibility that certain chronic infections are directly or indirectly related causally to atherosclerosis and atherosclerotic heart disease. This evidence may be summarized as follows:

(*a*) Chronic cardiac failure on the basis of atherosclerosis, unrelieved by the usual methods of treating cardiac failure will, at times, respond quickly to the removal of certain infections, particularly those of the gallbladder and the paranasal sinuses.[1]

(*b*) In chronic hyperplastic disease of the paranasal sinuses the living tissues removed at operation and immediately fixed in suitable fixing reagents show marked arteriolar sclerosis and thrombosis of the small deep-lying arteries, and micro-organisms, chiefly diplococci in character, can be recognized both in the walls of the vessels and in the thrombotic material in suitably stained sections. These vessel changes are seldom found in the sinus tissues of patients who have, for instance, chronic infectious asthma or rheumatoid arthritis. This fact suggests an "elective" affinity of these micro-organisms for vascular tissue.[2]

(*c*) Anatomically, a pathway of infection has been demonstrated which leads directly from the paranasal sinuses and the paralaryngeal lymph nodes into the systemic circulation of the body via the paratracheal lymphatic vessels to the great veins and thence into the right side of the heart and into the pulmonary bed. Thence micro-organisms may reach the pulmonary tissues or be carried by the blood stream to

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1. Jones, N. W., and Rogers, A. L.: Ann. Int. Med. **8**:834, 1935. (Four additional references are cited.)

2. Kistner, F. B.: Ann. Otol., Rhin. & Laryng. **43**:89, 1934. Jones and Rogers.[1]

other parts of the body. Experimentally, streptococci have been followed through this pathway into the spleen and the liver.[3]

(*d*) In the majority of cases the coronary thrombosis seen in man is on the basis of atherosclerosis.[4a] Micro-organisms, chiefly in the form of diplococci, have been found, by suitable staining methods, in the vessel wall and in the thrombus and have also been cultured.[2] These findings are subject to the criticism that postmortem tissues were used. Such criticism does not hold in the case of sinus tissues removed at operation.

(*e*) Experimental arteriosclerosis has been produced in rabbits by repeated intravenous inoculations of streptococci obtained from a coronary thrombus in man immediately after death. When cholesterol feedings were added to the inoculations in some of the litter rabbits, the arteriosclerotic lesions were more marked in those animals than when either streptococcic inoculations or cholesterol feedings were given alone.[4b]

(*f*) The lesions of arteriosclerosis in general are fundamentally both inflammatory and degenerative in nature, as attested by the studies of many investigators. One may cite the work of Ophüls, Klotz, Von Glahn, Pappenheimer, Clawson, Winternitz, to mention but a few. These men have expressed the belief that infection may play a role in the causation of the disease.

In order to verify, if possible, some of the aforementioned experimental findings and to see whether streptococci experimentally introduced into the paralaryngeal lymph nodes of the cat could be identified in the walls of the aorta and the coronary arteries, which would mean that they had gained entrance by way of the vasa vasorum of these vessels, we have conducted the following experiments.

EXPERIMENTS

Experiment 1.—Hyperplastic antral tissues removed from a patient who had atherosclerotic heart disease were pulverized and cultured in dextrose-brain-heart broth. Three different streptococci were obtained. The predominant one was a hemolytic diplostreptococcus occurring singly or in chains of two to eight pairs. The second organism was a small nonhemolytic streptococcus which occurred in short chains. The third was a small streptococcus which resembled a bacillus with irregular staining properties. This organism in this particular form was not recovered from animals into which it had been injected, a fact which might suggest that it had changed morphologically during the repeated culturing and animal passage. In the original cultures it showed slight hemolysis on blood agar.

A washed saline suspension of cultures of these three streptococci in a concentration of approximately 1 billion organisms per cubic centimeter was injected

3. Larsell, O.; Veazie, L., and Fenton, R. A.: Arch. Otolaryng. **27**:143, 1938.

4. (*a*) Benson, R. L., and Hunter, W. C.: Northwest Med. **24**:606, 1925. (*b*) Benson, R. L.; Smith, K. G., and Semenov, H.: Arch. Path. **12**:924, 1931.

into the paralaryngeal lymph nodes of 8 cats. Twenty hours later 2 cats were killed and cultures made from heart, aorta, coronary vessels, lungs, spleen and liver. Hartley digest broth was used in order to facilitate the growth of the streptococci. Two cats were killed after twenty-four hours and similar cultures obtained. About fifty-two hours after the first injection the remaining 4 cats were given an injection of a washed saline suspension of the same organisms in a concentration of approximately 5 billion per cubic centimeter. Nineteen hours later these 4 cats were killed and cultures from blood, heart, aorta, coronary vessels, lungs, spleen and liver were made in Hartley broth.

TABLE 1.—*Experiments on Cats*

Cat	Weight, Lb.	Anesthetic (Solution of Pentobarbital Sodium), Cc.	Injection of Culture			Time at Which Animal Was Killed, Hours After Injection	Gross Anatomy
			Side	Cc.	Concentration		
J-1	7½ (3.5 Kg.)	1.5	R L	0.2 0.2	1 billion	74½	Purulent nasal discharge, enlarged paralaryngeal nodes, acute splenic tumor
		1.5	R L	0.25 0.71	5 billion		
J-2	7 (3 Kg.)	1.15	R L	0.2 0.2	1 billion	24½	Purulent nasal discharge, early pneumonia, acute splenic tumor
J-3	6 (3 Kg.)	1.2	R L	0.2 0.2	1 billion	75	Enlarged paralaryngeal nodes
		2.0	R L	0.25 0.5	5 billion		
J-4	10 (4.5 Kg.)	1.9	R L	0.3 0.2	1 billion	73¼	Enlarged paralaryngeal nodes
		2.5	R L	0.5 0.25	5 billion		
J-5	7½ (3.5 Kg.)	1.5	R L	0.15 0.2	1 billion	74½	Enlarged paralaryngeal nodes
		2.3	R L	0.3 0.29	5 billion		
J-6	7 (3 Kg.)	1.4	R L	0.2 0.15	1 billion	20¾	Swollen paralaryngeal nodes, acute splenic tumor
J-7	10 (4.5 Kg.)	2.5	R L	0.5 0.65	1 billion	23½	Swollen paralaryngeal nodes, acute splenic tumor
J-8	5 (2 Kg.)	1.0	R L	0.15 0.35	1 billion	22¾	Acute splenic tumor

Smears from these cultures, made after thirty-six hours, revealed the organisms detailed in table 2.

It is to be noted that apparently the same diplostreptococcus and the same nonhemolytic streptococcus used in the inoculations were obtained six times and five times, respectively, in culturing aortic tissues and an equal number of times in culturing coronary artery tissues, although not uniformly in the same animals. Tissues from the aorta, the coronary arteries and the myocardium of cats J-5 and J-8 were fixed in Zenker solution to which formaldehyde solution U.S.P. had been added and sections stained for gram-positive bacteria. Sections of the paralaryngeal lymph nodes of these 2 animals were also stained in like manner and used as controls. Many of the lymph gland cells were filled with recognizable micro-organisms, some of which were evidently already undergoing phagocytosis. Similar phagocytic cells were demonstrated sparsely in all of the layers of the aortas

and coronary vessels extending from beneath the adventitia to the subintimal spaces. Such cells were not found in the myocardium. It may be noted also that the culture of the aortic tissue of cat J-5 remained sterile, but micro-organisms were found

TABLE 2.—*Cultures*

Tissue	Organisms	J Cats 1	2	3	4	5	6	7	8
Aorta	Diplostreptococcus	+	+	+*	+*	..	..	+	+
	Nonhemolytic streptococcus	..	+	+	+	..	..	+	+
	Gram-positive bacillus	+*	..	..	+	..	+	+	..
	Spore-bearing bacillus	..	..	..	..	..	..	..	+
	Sterile	..	..	..	..	+	..	..	..
Coronary arteries	Diplostreptococcus	+*	+	+*	+	+*	..	+*	..
	Nonhemolytic streptococcus	+	..	+	+	+	..	+	..
	Gram-positive bacillus	+	+	+	..	..	..	..	..
	Spore-bearing bacillus	..	+	..	..	+	..	..	..
	Staphylococcus	..	..	..	+	..	..	..	..
	Sterile	..	..	..	..	..	+	..	+
Myocardium with coronary artery	Diplostreptococcus	..	+	+*	+	..	..	..	+
	Nonhemolytic streptococcus	..	+	+	+	..	..	..	..
	Gram-positive bacillus	..	..	+	+	..	+	+	+
	Spore-bearing bacillus	..	..	..	..	+	..	..	..
Heart blood	Sterile	+	+	+	+	+	+	+	+
Lung	Diplostreptococcus	+	..	+	+	+	+*	..	..
	Nonhemolytic streptococcus	..	..	..	+	..	+	..	..
	Gram-positive bacillus	+	..	+	+	..	+	+	+
	Gram-negative bacillus	..	..	..	..	..	..	..	+
	Spore-bearing bacillus	..	+	..	..	..	..	..	..
Liver	Diplostreptococcus	+	..	+*	+*	..	..	+	+
	Nonhemolytic streptococcus	..	..	+	+	..	..	+	+
	Gram-positive bacillus	..	+	+	+	+	+	..	+
	Gram-negative bacillus	..	+	..	..	..	+	..	..
Spleen	Diplostreptococcus	+	+	+	+	+	..	+	+
	Nonhemolytic streptococcus	+	+	..	..	..	..	..	+
	Gram-positive bacillus	..	..	+	+*	+	..	+	..
	Gram-negative bacillus	+	..	..	..	..	..	..	..
	Spore-bearing bacillus	+	+	..	..	..	..	..	..
	Sterile	..	..	..	..	..	+	..	..

* This organism was predominant.

TABLE 3.—*Infected Tissues*

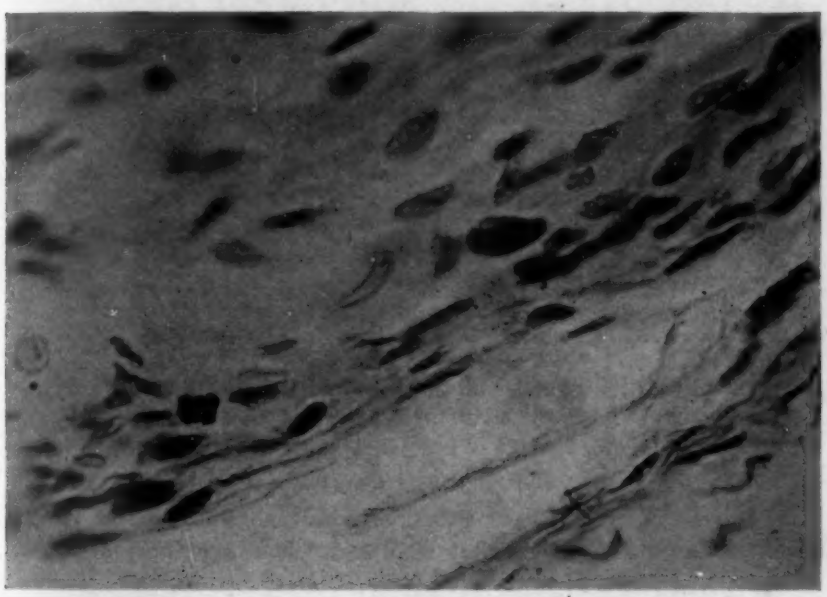
Cat	Aorta	Coronary Arteries	Myocardium	Heart Blood
J-1	+	+	+	0
J-2	+	+	+	0
J-3	+	+	+	0
J-4	+	+	+	0
J-5	0	+	0	0
J-6	0	0	0	0
J-7	+	+	0	0
J-8	+	0	+	0

in the stained sections. The culture of the coronary tissue of cat J-8 was accidentally lost.

Experiment 2.—An attempt was made to verify the experimental work of Benson, Smith and Semenov [4b] and produce arteriosclerotic lesions of the aorta or the coronary arteries or both by repeatedly inoculating rabbits intravenously with cultures of the same streptococci that were used in the first experiment. These inocu-

lations were given daily to 12 rabbits over a period of from six to twelve months. In no instance were lesions produced in these vessels. On the other hand, all of the animals showed multiple inflammatory changes of joints and muscles. The possibility of our having used micro-organisms with "elective" affinity for these tissues rather than for the blood vessels was considered.

Experiment 3.—In order to show clearly that particulate matter could be transported from the paralaryngeal lymph nodes by the blood stream to lodge in the walls of the aorta and the coronary vessels by way of the vasa vasorum, we injected subcutaneously a solution of trypan blue into the neck tissues about these lymph nodes in 2 white mice and 1 cat. Three injections of 1 per cent trypan blue in sterile isotonic solution of sodium chloride were given each animal at intervals of two days. The animals were killed five days after the first injection. The hearts were fixed in Zenker solution plus formaldehyde solution U.S.P. and sections were stained with safranine. Phagocytes laden with trypan blue granules



Photomicrograph of the base of the aorta of a cat, showing phagocytes laden with granules of trypan blue in the wall. The section is stained with safranine.

were demonstrated in the adventitia of the base of the aorta and of the coronary arteries of each of the white mice and lying deeper in the wall of the base of the aorta of the cat (figure). The presence of these granules clearly showed that particulate matter could enter by way of the vasa vasorum from the circulating blood. Petroff [5] injected trypan blue into rats and rabbits and found that it entered vessel walls through the endothelial lining as well as by way of the vasa vasorum.

Sections were cut from the paraffin blocks removed from a representative rabbit in the series of experiments conducted by Benson, Smith and Semenov [4b] and were stained for gram-positive bacteria. We used the aortic tissues of rabbit 3, litter 6, illustrated in their report. We were unable to demonstrate micro-organisms in the stained sections of these tissues.

5. Petroff, cited by Hueper.[7b]

COMMENT

Reference has been made in the literature from time to time by clinicians to the possibility that certain infections, particularly infections of the gallbladder, are related to vascular disease. The following instances of such clinical cases are to the point.

(*a*) A 59 year old business man had been confined to bed because of cardiac failure, which was on the basis of atherosclerotic heart disease, for three months before entering the hospital. It was not possible to recompensate his heart by the usual therapeutic measures. Removal of the chronically hyperplastic, diseased tissues of the antrums permitted a rapid compensation to take place. The patient returned to his business, was well for a year and a half and died suddenly of acute coronary thrombosis.

(*b*) A 49 year old housewife had suffered from congestive heart failure that was on the basis of atherosclerotic heart disease with hypertension and auricular fibrillation, for two years and had been confined to bed for six months. She had undergone cholecystectomy some years before, but subsequently attacks of pain of the right upper quadrant of the abdomen, slight chills and fever, and fleeting jaundice had returned. Recompensation of the heart proved to be impossible under hospital control, so that drainage of the common bile duct was done in spite of the severe congestive failure. Convalescence was not stormy. The heart became compensated easily, the auricular fibrillation was returned to normal sinus rhythm with quinidine, and the patient lived for fifteen years in ordinarily good health. The final cause of her death was not learned.

(*c*) A 62 year old lawyer had suffered shortness of breath on slight exertion for six years. For three years he had spent much time in bed. His heart, although not greatly enlarged, suggested the atherosclerotic type of disease and a right bundle branch block. He had chronic hyperplastic pansinusitis. The diseased tissues were bilaterally removed by radical antrotomy and transantral ethmosphenoidectomy. Symptomatic recovery was prompt. He returned to his professional work and lived for seven years. He died of congestive heart failure induced by an unrelieved attack of bronchial asthma.

Arteriosclerosis has been produced in 3 month old chickens by cholesterol feeding.[6] Of greater significance has been the noteworthy work of Hueper.[7a] He found that the injection of various colloidal solutions of macromolecular carbohydrates, such as polyvinyl alcohol, methyl cellulose, sodium cellulose glycollate and pectin, produced intimal degenerative and calcifying reactions in the aorta and the large and middle-sized arteries of dogs as well as of rabbits. He stated that atheromatous lesions are found exclusively with colloidal disturbances of the lipoid substances and carbohydrates of the plasma.[7b, d] Cavelti [8] has produced glomerulonephritis in rats by the use of a mixture of killed group A

6. Dauber, D. V., and Katz, L. N.: Arch. Path. **34**:937, 1942; **36**:473, 1943.

7. Hueper, W. C.: (*a*) Arch. Path. **39**:51, 1945; (*b*) **39**:117, 1945; (*c*) **39**: 187, 1945 (7 additional references cited); (*d*) Biol. Symposia **11**:1, 1945.

8. Cavelti, P. A.: Proc. Soc. Exper. Biol. & Med. **60**:379, 1945. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **39**:148, 1945; **40**:158 and 163, 1945.

streptococci and emulsion of rat kidney tissue of the same species. The autoantibodies thus formed acted as a pathogenic agent for kidney. In the same manner, lesions of the heart and connective tissues, quite similar to those of acute rheumatic fever, were produced when the animals were immunized with an emulsion of heart tissue.

In view of the clinical and experimental facts just enumerated, might it not be justifiable to look on certain chronic infections with a possible "elective" affinity for vascular tissues as atheromatogenic agents which, operating through the years, may, by disturbing the nutritive and oxidative metabolism of the vascular walls, be an important factor in the genesis of the disease; or, on the other hand, may it not be due to the pathogenic action of specific blood vessel autoantibodies, as the work of Cavelti [8] might suggest? It would seem that these phases of the problem deserve further study.

SUMMARY AND CONCLUSIONS

The clinical course of some patients suffering from cardiac failure that was on the basis of atherosclerotic heart disease responded favorably to the removal of certain chronic infections.

A direct anatomic pathway was demonstrated to exist between the paranasal sinuses and the paralaryngeal lymph nodes, which drain these sinuses, and the systemic circulation. Micro-organisms introduced into these sinuses or lymph nodes were recovered by culture from the walls of the aorta and the coronary arteries, as well as from other organs, and were demonstrated in stained sections of these tissues.

Particulate matter in the form of trypan blue granules introduced into the paralaryngeal lymph nodes were demonstrated in the phagocytic cells within the wall of the aorta and coronary arteries.

In the sections of the thickened, thrombosed small arteries of chronically hyperplastic sinus tissues removed at operation micro-organisms were demonstrated. Likewise, in the sections of some of the thrombosed coronary arteries obtained at autopsy from patients dying of acute coronary thrombosis similar organisms were demonstrated.

In view of the facts given it is suggested that such chronic infections might well be studied from the standpoint of their being a direct or an indirect agent in the cause of atherosclerosis.

FAT NECROSIS OF THE NEWBORN

Report of a Case with Necrosis of the Subcutaneous and Visceral Fat

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THIS is the report of the case of an infant in whom subcutaneous fat necrosis developed shortly after birth. Subsequently, severe enteritis developed, and the infant died at the age of 2 months. At autopsy, in addition to the expected necrosis of subcutaneous fat, the same process was found in the perirenal fat and that about the ribs. Although many cases of subcutaneous fat necrosis have been reported,[1] the majority of those reporting these cases concern themselves with clinical observations and biopsies of the skin and subcutaneous tissues. A search of the literature has revealed only 3 cases[2] similar to the present one.

REPORT OF CASE

On March 8, 1943, a white boy weighing 3,650 Gm. was born in the New York Hospital after a labor of fifty-six hours. The mother was 38 years of age and of Puerto Rican extraction. One pregnancy, in 1922, resulted in a normal baby, and two others, in 1941, terminated as miscarriages at two and three months. The mother was first seen in the prenatal clinics of this hospital on Oct. 27, 1942, when she was about five months pregnant. The blood Wassermann reaction was negative, and the blood pressure was 118 systolic and 70 diastolic. By Feb. 22, 1943, her blood pressure was 150 systolic and 100 diastolic, and the urine contained some albumin. Slight edema of the subcutaneous tissues developed, and the mother was admitted to the hospital on February 26 with a diagnosis of mild toxemia of pregnancy with edema and hypertension. Examination revealed that she was also suffering from an acute infection of the upper respiratory tract with acute left frontal sinusitis. Her blood pressure was 140 systolic and 96 diastolic, and she was given a low salt diet and kept in bed. The pelvis was of an android type with a narrow arch.

Labor began on March 6 at 2 a.m. and was slow at first. After thirty-two hours the cervix was found to be thick and tough, and its dilatation was slow, continuing for another twenty-three hours. The second stage was not difficult,

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1. (*a*) Fox, H.: Arch. Dermat. & Syph. **27**:237, 1933. (*b*) McIntosh, J. F.; Waugh, T. R., and Ross, S. G.: Am. J. Dis. Child. **55**:112, 1938.

2. (*a*) Zeek, P., and Madden, E. M.: Arch. Path. **41**:166, 1946. (*b*) Kohnstam, G. L. S., and Herbert, F. K.: Arch. Dis. Childhood **2**:349, 1927. (*c*) de Bruin, M.: Nederl. tijdschr. v. geneesk. **1**:1221, 1929.

and on March 8 the head and then the body were delivered without undue trauma. The total duration of labor was fifty-six hours. The subsequent recovery of the mother was uneventful.

After birth the infant breathed without difficulty, and the initial examination disclosed nothing remarkable save for marked molding of the head. The infant appeared normal until the day after birth, March 9, when he cried frequently as if in pain, and a mass was found in the subcutaneous tissues of the back of the neck. Detailed examination showed that the baby was well hydrated and the head well formed except for a caput succedaneum over the vertex. Extending from the occipital region of the head over the posterior portion of the neck to the lower portion of the back was a diffuse swelling, which had a brawny character but pitted on pressure. The involved area was not warm. Treatment was begun with warm compresses. On March 10, two days after delivery, the edema of the tissues of the back had increased and was noted to be indurated. A diagnosis of subcutaneous fat necrosis following trauma was suggested. The baby took its feedings poorly and had a low grade fever, the temperature reaching 38.8 C. (101.8 F.). By March 13 the swelling had ceased to increase, and by March 16 it had begun to subside. Because of the continuance of the low grade fever and the difficulty of feeding, the child was transferred to the pediatric service.

On pediatric examination, March 17, the boggy indurated area of the skin and connective tissues of the posterior aspect of the chest and neck was still present. It had a "lumpy feel"; no discoloration of the skin was noted. The impression was fat necrosis of the subcutaneous tissues and a mild infection of the upper respiratory tract. It was thought that the absorption of necrotic fat contributed to the maintenance of the low grade fever. The child was given 0.1 Gm. of sulfadiazine six times daily, and by the fifth day of this treatment the temperature fell to normal. On March 17 the hemoglobin was 12 Gm., the red blood cell count 4,200,000, and the white blood cell count 20,100. By March 23 the latter had fallen to 13,200, with a differential count of 62 polymorphonuclear leukocytes, 3 eosinophils, 2 monocytes and 32 lymphocytes per hundred cells. The Kline test for syphilis was negative. The urine contained no sugar and only a faint trace of albumin. Soon the patient was taking food well and was active. Numerous nodules could still be felt in the subcutaneous tissues of the back. Since it remained afebrile, the infant was discharged on March 27.

On the first visit to the clinic, April 2, the infant seemed well, but was readmitted to the New York Hospital on April 19. At this time the infant was small, poorly nourished and dehydrated. The temperature was 36.2 C. (97.2 F.) and the weight only 3,100 Gm. The infant was emaciated. In the posterior cervical and intracapsular regions of the back the skin was indurated and hard but not warmer than normal. Examination of the infant otherwise disclosed nothing remarkable. The stools were numerous, large and light green, and contained much slimy mucus and water. The impression was parenteral diarrhea and healing subcutaneous fat necrosis of the back. On two occasions, cultures of material taken from the nasopharynx and throat contained hemolytic Staphylococcus aureus, as did the stool on one occasion. The stool contained no organisms of the typhoid, paratyphoid or dysentery groups. Administration of sulfadiazine had little effect on the diarrhea, which continued. Despite transfusions of whole blood, injections of sodium lactate, infusions and other treatments, the infant failed to improve, lost more weight, and died on May 13, at the age of 66 days.

The final clinical diagnosis was: parenteral diarrhea, acidosis, acute nasopharyngitis and healing subcutaneous fat necrosis.

Gross Anatomic Findings.—The body was that of a markedly emaciated white boy; it weighed 2,990 Gm. The extremities were thin, and the skin of the entire body was loose. There was no subcutaneous edema. Over the back was a definite thickening of the subcutaneous tissues over the midportion of the lumbar region. When an incision was made in this area, the subcutaneous tissues appeared slightly firmer than normal, and the fat was peculiarly firm and white. The fat of the anterior abdominal wall was almost gone and was replaced by rather loose connective tissue. No fat was visible in the greater omentum, in the mesentery of the intestines or in the tissues of the mediastinum and the neck. The fat about the kidneys was abundant, of a peculiar whitish yellow color, and definitely firmer than normal. It formed a large fat pad, well preserved, in contradistinction to the general lack of bodily fat in the emaciated tissues. There were scattered discrete nodules of this peculiar whitish fat elsewhere in the retroperitoneal tissues and in the large fat pads of the lower lumbar portion of the back. Similar white, firm fat was also seen in the subcutaneous tissues of the chest and about the ribs.

The visceral organs were carefully examined. The thymus was small, weighing only 4.5 Gm., and on cut surfaces was extremely soft. The heart, the great vessels and the lungs showed no abnormalities. The spleen weighed 15 Gm., and the cut surfaces were moderately firm. The liver weighed 125 Gm., and its cut surface was purple-red. The gallbladder, and the common, cystic and hepatic ducts were patent. The pancreas was of normal size and shape, and the cut surfaces showed uniform pinkish pancreatic tissue. The adrenal glands were likewise of normal size, and on cut surfaces the usual thin layer of cortex was seen. The kidneys themselves were not enlarged, and not altered on cut surfaces. The bones, the brain, the organs of the neck and the thyroid gland were of normal appearance.

In the gross the esophageal mucosa was smooth, as was that of the stomach. The jejunum and the ileum contained some yellowish liquid material, and their mucosa appeared slightly hyperemic but not granular. There was no visible ulceration on the surface of the small intestine. The appendix was of normal size and unremarkable. The colon contained some soft or liquid yellowish stool, and the mucosal surfaces were pale. The mesenteric lymph nodes were enlarged up to 10 by 3 by 3 mm. Their cut surfaces showed fleshy pink tissue. Other lymph nodes were not enlarged.

Postmortem cultures were made of the heart's blood, which yielded only a gram-negative bacillus, presumably saprophytic. Cultures of the colonic content showed Monilia albicans and Bacillus coli but no members of the typhoid or the paratyphoid group. Cultures of the content of the lower part of the ileum showed nonhemolytic Staphylococcus aureus and nonhemolytic Streptococcus, alpha prime and gamma.

Microscopic Observations.—In sections through the skin and subcutaneous tissues of the upper part of the back, the skin was normal. The subcutaneous layer of fat was, however, markedly altered (fig. 1). The entire mass of fat was necrotic, but the cell outlines were, for the most part, well preserved. As seen in the hematoxylin-eosin stain, many of the fat cells were filled with slitlike spaces suggesting crystalline structures within the cells. In a section stained for fat with sudan III these cells contained much lipoid material, most of which definitely had the appearance of needles or thin plates. In some places the fat had escaped from the cells, and large vacuoles were present in or near the mass of fat. In the periphery of the fat lobules were large giant cells, small giant

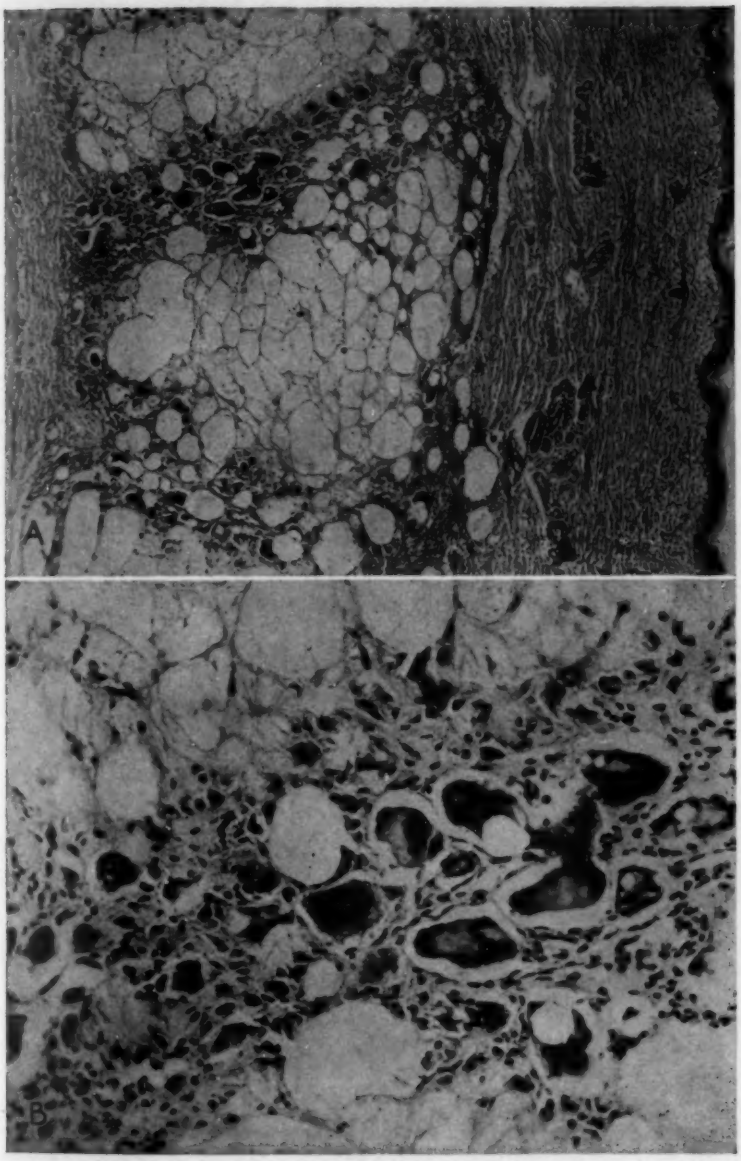

Fig. 1.—*A*, section through the skin and subcutaneous fat of the back. The fat cells are necrotic, and in places the free fat is forming small cystlike spaces. The connective tissue reaction elicited by the altered fat is confined to the fat pad; the adjacent tissues are unaltered. Hematoxylin-eosin stain; × 50.

B, high power magnification of a part of *A*. Giant cells and acicular spaces can be seen in the necrotic fat. Hematoxylin-eosin stain; × 180.

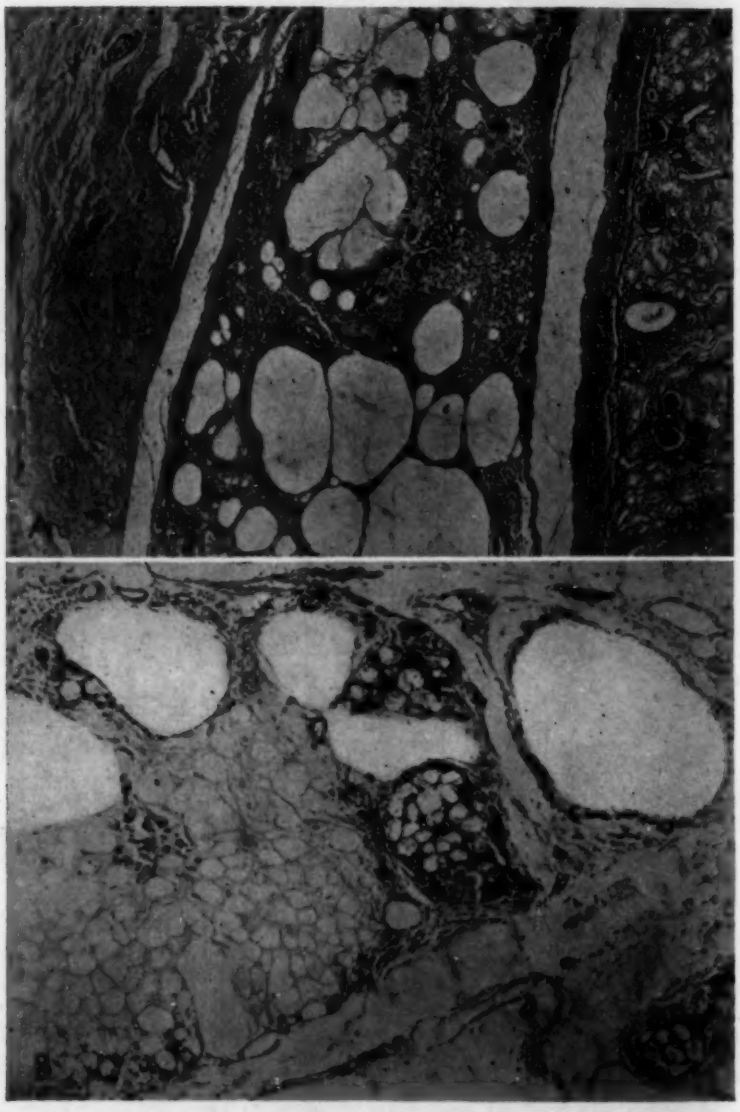

Fig. 2.—*A*, section through the perirenal fat. The necrotic fat cells are breaking down, and the free fat has formed cystlike spaces, surrounded by giant cells, fat-filled macrophages and fibroblasts. Both the capsule of the kidney and the connective tissue peripheral to the affected fat are thickened. The longitudinal space between the fat mass and the kidney and that between the fat and the connective tissue are artefacts. Hematoxylin-eosin stain; × 50.

B, section through the fat about a rib, stained by von Kossa's method for calcium. Some of the necrotic fat cells are intact; others have degenerated, and the free fat is occupying cystlike spaces. The black material in the central region is calcified necrotic fat. × 90.

cells and many fat-filled macrophages. There was only a slight increase of connective tissue about the mass of necrotic fat, and it seemed of recent origin. A section of the fat pads in the lumbar region of the back was similar.

The perirenal fat (fig. 2*A*) showed areas in which the fat tissue was necrotic and in which the outlines of the fat cells could be seen. In these necrotic cells were laminated structures of a crystalline form. In some areas of the perirenal fat, however, the actual structure of the fat was lost, with only vacuolated spaces remaining, in some of which were laminated areas suggestive of crystals and about which were many large giant cells, as well as macrophages filled with fat. The connective tissue both above and below the necrotic perirenal fat was moderately thickened. The necrotic fat about the ribs was similar to that about the kidneys and in the subcutaneous tissues, except for areas of calcification (fig. 2*B*).

In examining other organs, special attention was paid to the adjacent fat. In all areas where the fat cells were of the "fetal" type, that is, relatively small and

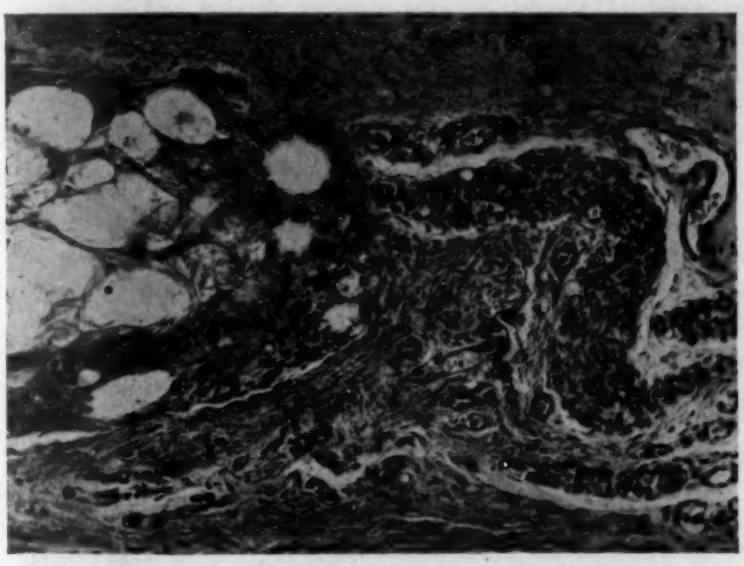

Fig. 3.—Section through the perirenal fat. One area of fat necrosis is visible, and, adjacent to it, an uninvolved area of fetal fat. Hematoxylin-eosin stain; × 180.

containing small cytoplasmic vacuoles, there was no fat necrosis. This was observed in the atrophic epicardial fat, in lobules of fetal fat adjacent to the pancreas and the adrenal glands and even in fetal fat in the capsule of the kidney, some of which was adjacent to areas of fat necrosis (fig. 3). Normal fetal fat tissue was also seen about the thyroid gland, in the mesentery and even in a few areas of subcutaneous tissue. In the bone marrow itself there was no fat.

The histologic examination of the heart, the lungs, the trachea, the liver and the spleen revealed nothing remarkable. In the pancreas the parenchymal glands were well preserved. The islets themselves appeared unremarkable in some instances. In a few of the islets, however, the cells appeared slightly larger than normal and had somewhat hyperchromatic nuclei. In the adrenal glands the subcortical zone was represented only as a very thin band of tissue; the definitive cortex and medullary tissue appeared normal. In the kidneys the glomeruli

appeared normal; some of the tubular cells were slightly swollen. The prostate and the testes were infantile. The thyroid gland was normal. The bone marrow of the tibia was rather hyperplastic and contained many neutrophilic leukocytes and their precursors. Sections of the diaphragm and of the iliopsoas muscle were unremarkable. The gastric mucosa was atrophic, with very few acid-forming cells in the glands, and a diffuse infiltration of plasma cells, macrophages and scattered eosinophilic leukocytes. The duodenum was normal. In the jejunum the mucosa was intact, but in the villi were many scattered plasma cells and other mononuclear cells, as well as a few eosinophils. In the submucosa of the ileum, however, were many plasma cells and an infiltration of macrophages, lymphocytes and scattered eosinophils. One section of ileum contained a superficial ulcer. In the colon the glands were essentially normal, but between them were a few plasma cells and eosinophils. The mesenteric lymph nodes were markedly enlarged, and the sinusoidal structure was very prominent. The nodes were filled with lymphocytes and macrophages. The germinal centers were not abundant, and in the centers of some were focal areas of necrosis.

Anatomic Diagnosis.—Subacute enteritis with focal ulcers of the ileum and mesenteric lymphadenitis; generalized necrosis of subcutaneous and visceral fat; marked emaciation.

COMMENT

The attack of subcutaneous fat necrosis of the newborn described was not of unusual severity. If the infant had not succumbed to another disease, the changes in the visceral fat, which in both the gross and the microscopic examination were similar to those in the subcutaneous fat, would not have been discovered. At least 3 similar cases have been described. The first was that of de Bruin,[2c] who observed necrosis of perinephric fat associated with subcutaneous fat necrosis of the newborn. The second was described by Kohnstam and Herbert[2b]: An infant died of what they called sclerema neonatorum. It had had nodules and areas of induration over most of the skin and subcutaneous tissues, and died at 6 weeks of age. The microscopic examination of the subcutaneous fat revealed fat necrosis, with acicular crystals, and an inflammatory reaction. In their gross description they commented on the fact that the changes in the subcutaneous fat were identical with those about the kidney, stating that the "perinephric fat was small in amount, but was also white in color." The third case was recently reported by Zeek and Madden.[2a] In their case the clinical course was that of sclerema neonatorum in that, when the patient was examined on the ninth day of life, there was hard, nonpitting edema of almost the entire subcutaneous tissue save for that of the palms, the soles and the genitalia. The temperature was subnormal, and the child died on the sixteenth day of life. At autopsy there were degeneration and necrosis of the subcutaneous and visceral fat, the latter including fat about the thymus, the root of the diaphragm, the adrenal glands and the aorta and that in the mesentery and about the bronchi. The histologic changes in the necrotic fat were similar to those described in the present paper, but there was

somewhat more proliferation of fibrous tissue in and around the fat lobules. They noted that the lesions were histologically typical of fat necrosis of the newborn.

The problem of the classification of these 4 cases is an interesting and difficult one, and is complicated by the confusion of terminology which now exists in the literature. In the United States and in continental Europe "subcutaneous fat necrosis," or "adiponecrosis subcutanea neonatorum," is usually considered to be a self-limited process with few constitutional symptoms. Fox [1a] stated that the lesions are first noticed at birth or within the first two or three weeks of life and vary from small subcutaneous nodules to extensive lesions beneath the skin. Complete recovery occurs in a few months. The essential process consists in necrosis of subcutaneous fat cells with a granulomatous infiltration of cells, among which giant cells predominate, and with the presence of needle-like crystals in the necrotic fat cells. His views are substantiated by others.[3]

Sclerema neonatorum is a poorly defined disease. Most authors [4] have described it as a usually fatal disease, appearing between birth and the tenth day of life, in which the entire skin, the subcutaneous fat and the connective tissue, except that of the palms, the soles and the scrotum, become leathery and assume the hue of wax. The temperature becomes subnormal, and death ensues. Congenitally affected subjects have been described, and such infants may be stillborn.

The pathologic changes in sclerema neonatorum are variable. In a frequently quoted paper, that of Gray,[5] this disease is differentiated into two types. The first type is, surprisingly enough, what is referred to in the United States as "subcutaneous fat necrosis," as Gray stated clearly in his discussion. He described the clinical and pathologic observations in 2 fatal cases of this disease and in a case in which the infant died of enteritis. In all, the subcutaneous fat was chalky white, of a peculiar lard-like consistency, and in places there were cystlike spaces in the subcutaneous tissues. Histologic examination of the subcutaneous fat revealed disintegration of liquid fat in the cells and invasion of the fat lobules by many fibroblastic cells and numerous giant cells. Many crystals were seen in the uninvaded fat cells and in the areas containing giant cells. Calcification was also noted. The changes illustrated in his photomicrographs of these tissues are similar to those that have been observed in

3. Grütz, O.: Arch. f. Kinderh. **113**:200, 1938. Bernheim-Karrer, J.: Ztschr. f. Kinderh. **55**:695, 1933. McIntosh and others.[1b]

4. (*a*) Ormsby, O. S.: Diseases of the Skin, ed. 4, Philadelphia, Lea & Febiger, 1934, pp. 548 and 550-551. (*b*) MacKee, G. M., and Cipollaro, A. C.: Skin Diseases in Children, ed. 2, New York, Paul B. Hoeber, Inc., 1946, pp. 256 and 276.

5. Gray, A. M. H.: Arch. Dermat. & Syph. **14**:635, 1926.

"subcutaneous fat necrosis." His clinical description of this disease is also the same, and because of the confusion which exists concerning his paper, this quotation is made:

> . . . The process of solidification of the fat is more or less gradual and almost invariably commences in the first few days of life. It is questionable whether it occurs after the first month, and, although a good number of cases have been described in which the condition was present at birth, it would be as well to reserve judgment until further investigations have been made on such cases. The disease attacks infants who generally appear healthy, whose temperature is not markedly subnormal, who, as often as not, are born of healthy parents and have received no injury at birth nor have been born with severe asphyxia. The regions chiefly affected are the cheeks, shoulders, scapular regions, arms, buttocks, thighs and calves. A considerable number of patients recover, and those who die frequently do so from some intercurrent malady.

He distinguished a second type of the disease:

> The second type of case appears to occur in weakly, premature or marasmic infants or in those who are more or less moribund from some severe wasting disease, especially from epidemic gastro-enteritis. The solidification of the fat occurs with much greater rapidity than in the former type and is much more diffuse, the whole of the body surface being affected, with the exception of the penis, scrotum, palms and soles. It may occur at any time during the first six months of life and is more common during the summer months.

In the case he described the autopsy revealed no changes in the fat beyond a slight increase of the cells between the trabeculae. He called this a case of "acute sclerema" and distinguished it sharply from the others.

As can be seen from Gray's paper, he did not separate his first group of cases of "sclerema neonatorum" from "subcutaneous fat necrosis of the newborn," stating with reference to his use of the term "sclerema neonatorum," "also to the same class belong the cases described recently as 'subcutaneous fat necrosis,' 'subcutaneous fat sclerosis,' 'adiponecrosis subcutanea neonatorum, etc."

It is therefore most surprising to find authorities—e. g., Ormsby [1a] and McCarthy [6]—who have sharply differentiated "sclerema neonatorum" from "subcutaneous fat necrosis of the newborn," citing the histologic observations of Gray [5] in sclerema neonatorum without realizing that he considered all as one disease entity, save only for the case of "acute sclerema."

The purpose of this rather lengthy discussion of terminology is to attempt to eliminate the confusion which originated not in the work of Gray,[5] which is clarity itself, but from those who have cited him.

McIntosh, Waugh and Ross,[1b] as did Gray,[5] would use the term "sclerema neonatorum" synonymously with "subcutaneous fat necrosis."

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6. McCarthy, L.: Histopathology of Skin Diseases, St. Louis, C. V. Mosby Company, 1931, p. 89.

They have suggested, however, that "preagonic induration" is best applied, rather than "sclerema," to designate "a diffuse hardening of the subcutaneus tissues occurring in weakly or marasmic infants, not necessarily new-born," which is apparently the disease that Gray [5] called "acute sclerema."

The exact terms used in describing the 3 previously observed cases of fat necrosis in which both the subcutaneous and the visceral fat of infants was involved have varied: "sclerema neonatorum," [2b] "sclerema adiposum neonatorum" [2a] and "fatty necrosis in the newborn" [5] have been used by the respective authors to designate what is probably the same disease. In the present case the clinical designation was "subcutaneous fat necrosis of the newborn."

How, then, may this matter of nomenclature be clarified? Should one use "subcutaneous fat necrosis" or "sclerema neonatorum" to describe a disease process in which, as indicated by 4 cases now described, there may be necrosis not only of the subcutaneous but also of the visceral fat?

The term "sclerema neonatorum" is used in a confusing fashion. Perhaps it would be better to restrict this term to what has been called "acute sclerema" [5] or "preagonic induration," [2] to describe a disease of infants characterized by diffuse hardening of the subcutaneous tissues occurring in cachectic infants in which there is no necrosis of fat. Then the term "fat necrosis of the newborn" could be used for all conditions in which this pathologic change was found. It is evident that the term "subcutaneous" should be dropped as a general descriptive adjective, since the disease may be present in both subcutaneous and visceral fat.

It is difficult to state what percentage of cases of "fat necrosis of the newborn" have involvement of both subcutaneous and visceral fat, but this evidently is either uncommon or else has been overlooked in some instances.

The cases of "fat necrosis of the newborn" in which necrosis of visceral as well as of subcutaneous fat is found are of interest when one considers the current theories [7] of the genesis of the disease, most of which are based on the concept that this is a disease of the subcutaneous tissues. Cold and trauma can scarcely be factors in necrosis of perinephric and other visceral fat. The suggestion of Zeek and Madden [2a] that "the most plausible explanation of the lesions in this infant seems to be that they were caused by some generalized disturbance of fat metabolism" applies to this case as well as to theirs. In their case there was

7. McIntosh and others.[1b] Zeek and Madden.[2a] Kohnstam and Herbert.[2b] Gray.[5]

some evidence that the origin of the disease may have antedated birth; evidence for such an origin was certainly lacking in the present case.

In the present case the fact that the disease process was not present in the "fetal fat" tissue of the body is subject to at least two interpretations. It is possible that this embryonic fat tissue actually contained little or no fat at the time of the onset of the disease and therefore was uninvolved; or, on the other hand, what appeared at autopsy to be "fetal fat" may simply have been fat which was not involved by the initial disease process and, as the diarrhea and emaciation developed, became secondarily atrophic. Perhaps some combination of the two possibilities actually occurred.

SUMMARY

A case has been reported of an infant in whom subcutaneous fat necrosis developed shortly after birth. Subsequently severe enteritis developed, and the patient died at the age of 2 months. At autopsy, in addition to the necrosis of the subcutaneous fat, with the presence of crystalline structures in necrotic fat cells, cyst formation and a reaction of macrophages, fibroblasts and giant cells, a similar pathologic process was found in the visceral fat about the kidneys and in that about the ribs.

The relationship of subcutaneous fat necrosis of the newborn, sclerema adiposum neonatorum, and sclerema neonatorum with necrosis of fat is discussed, and it is suggested that the term "fat necrosis of the newborn" be used for what is apparently a single disease entity.

HISTOPATHOLOGY OF VIRUS ENCEPHALOMYELITIS

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THE PROBLEMS concerning the distinctive classification of the numerous forms of encephalitis according to their morphologic features are still a matter of controversy and confusion. Efforts toward classification are frequently blocked by lack of sharp demarcation among the several forms of encephalitis. The difficulty of classification hinges also on lack of a clearcut interpretation of the underlying histopathologic changes.

The purpose of this presentation is to outline some of the pertinent histopathologic observations characteristic of encephalomyelitis caused by virus infection.

MATERIAL AND METHODS

This report is based on the study of 10 cases of acute anterior poliomyelitis, 1 case of Japanese, or type B, epidemic encephalitis and 1 case of Vienna (von Economo's), or type A, epidemic encephalitis.

Sections were taken from numerous regions of both hemispheres, the basal ganglions, the midbrain, the medulla oblongata, the cerebellum and the spinal cord and were stained with hematoxylin and eosin and cresyl violet and by the method of Loyez for myelin sheaths, the Bodian 1 per cent "protargol" (strong silver protein) method for nerve fibers and Hortega's silver carbonate method for microglia.

JAPANESE, OR TYPE B, EPIDEMIC ENCEPHALITIS

The histopathologic lesions in Japanese B encephalitis consist of two principal types of abnormalities: (1) ectodermal changes and (2) mesodermal reactions.

Ectodermal Reactions.—The ectodermal reactions are characterized by degenerative and proliferative alterations. The former relate to nerve cells, the degeneration and destruction of which are widespread; the latter, to microglial elements.

Degenerative Alterations: The degeneration of nerve cells is at its maximum throughout the cerebral cortex, the thalamus, the substantia nigra, the pons, floor of the fourth ventricle, the inferior olives, the dentate nuclei and the cerebellar cortex. The essential neuronal lesions are mostly focal in extent and are limited to a small group of nerve cells (figs. 1 and 2). Only occasionally, through the coalescence of several foci, may there be seen more extensive lesions involving several layers of the cerebral cortex. The widely disseminated focal lesions vary in size; some of them are confined to a few nerve cells, while others involve twenty or more neuronal elements.

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Various stages of neuronal damage are noted. Though spared from complete destruction, some of the ganglion cells display considerable swelling of both nucleus and cytoplasm; these changes are frequently associated with loss of stainability. In some of the nerve cells the nucleus is eccentrically placed and appears shrunken and darkly stained. The cell cytoplasm is often irregularly outlined, eaten away and invaded by glial nuclei. Many cells finally display signs of complete destruction. Occasionally, however, the nerve cells are found to be relatively well pre-

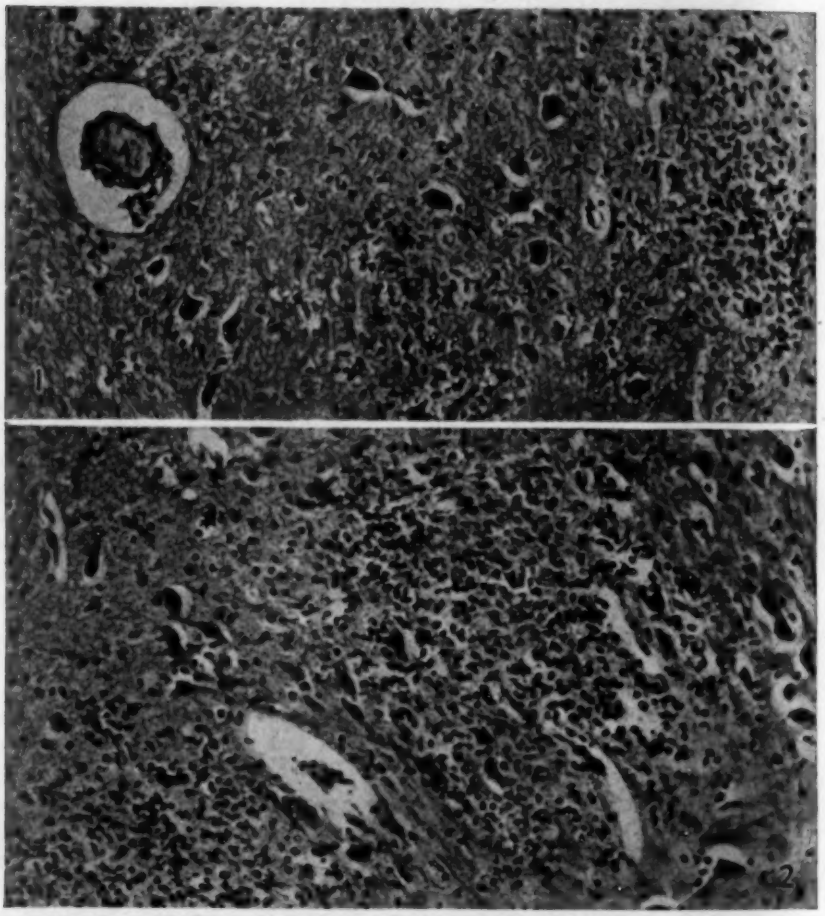

Fig. 1.—Japanese B encephalitis. Focal neuronal lesions limited to a small group of nerve cells. Note the proliferation of a large number of microglia cells. Hematoxylin-eosin; × 185.

Fig. 2.—Japanese B encephalitis. Coalescent foci of neuronal destruction with concomitant microglial proliferation. Hematoxylin-eosin; × 185.

served and merely surrounded, but not invaded, by glia cells. The latter are, as a rule, confined to the extremely distended pericellular spaces.

Far less frequent is another type of focal, circumscribed lesion, characterized by spongy transformation and complete destruction of the nervous parenchyma

(nerve cells, myelin sheaths and nerve fibers) (fig. 3). These spongy lesions are almost acellular and show slight degrees of reactive glial changes and absence of inflammatory reaction. None of the foci is related to blood vessels. These lesions are similar to those described by Zimmerman[1] as "acellular plaques."

Proliferative Alterations: Concomitant with neuronal destruction, there is striking proliferation of microglia cells. Under low power magnification the focal areas of neuronal destruction display the appearance of cellular "nodules" or "granulomas" (fig. 4). The bulk of each nodule is composed of a large number of proliferated microglia cells; their nuclei display a considerable polymorphism, frequently assuming rod-shaped, oval or irregular outlines. Occasionally there are a few cells resembling lymphocytes and a small number of large mononuclear elements with a spherical or ovoid nucleus and abundant cytoplasm. No astrocytes, gitter cells or polymorphonuclear leukocytes seem to participate in the cellular composition of the nodule. Most of the nodules seem to be formed around severely damaged or completely destroyed nerve cells and are widely scattered throughout the gray matter of the cortex and the basal ganglions. Their distribution is similar to that of the neuronal destruction. On cross sections they appear, as a rule, as round or ovoid bodies; only occasionally do they extend over larger areas of the cortex, embracing several laminas.

Mesodermal Reactions.—These occur in the smaller blood vessels of the cerebral tissue and in the leptomeninges, and they are characterized by perivascular accumulation of lymphocytes, monocytes and a few macrophages. The perivascular "cuffing" varies in degree and distribution. The smallest perivascular alterations consist in the accumulation of a few lymphocytes and monocytes within the perivascular space. Quite frequently, however, the lymphocytes and mononuclear elements are seen not only in the perivascular space but also in the vascular sheaths and in the adjacent nervous tissue. The nervous parenchyma adjacent to some of the blood vessels displays diffuse proliferation of microglia cells. In some areas an occasional typical transition from microglia cells to fat-containing gitter cells can be observed. Perivascular cuffing is widely distributed throughout the gray and the white matter of both hemispheres. In the white matter, however, this type of change is frequently observed in areas where other types are completely absent.

The leptomeninges show cellular infiltration of a moderate degree. The predominant cellular elements are lymphocytes, histiocytes and macrophages; larger collections of these cells are seen mostly around the smaller veins of the arachnoidea. Only relatively few cells (chiefly histiocytes) are to be seen scattered through the meshes of the subarachnoid space.

The over-all distribution of the leptomeningeal changes is irregular and patchy in character. Whereas some sections display no or little meningeal change, others show marked cellular infiltration. Over the midbrain, the pons, the medulla, the cerebellum and the spinal cord the leptomeningeal reaction is minimal.

ACUTE ANTERIOR POLIOMYELITIS

The histopathologic lesions characteristic of human poliomyelitis might be briefly summarized as follows: The neuronal alterations are the most predominant and striking features of the morbid process.

Spinal Cord.—The large majority of the anterior horn cells is destroyed and replaced by a large number of proliferated microglia cells (fig. 5). The neuronal

1. Zimmerman, H. M.: Arch. Path. **22**:965, 1946.

destruction is, as a rule, at its maximum at cervical and lumbar levels of the spinal cord. Nerve cells that are not entirely destroyed display various stages of degeneration. The cytoplasm of some of the nerve cells is irregularly outlined,

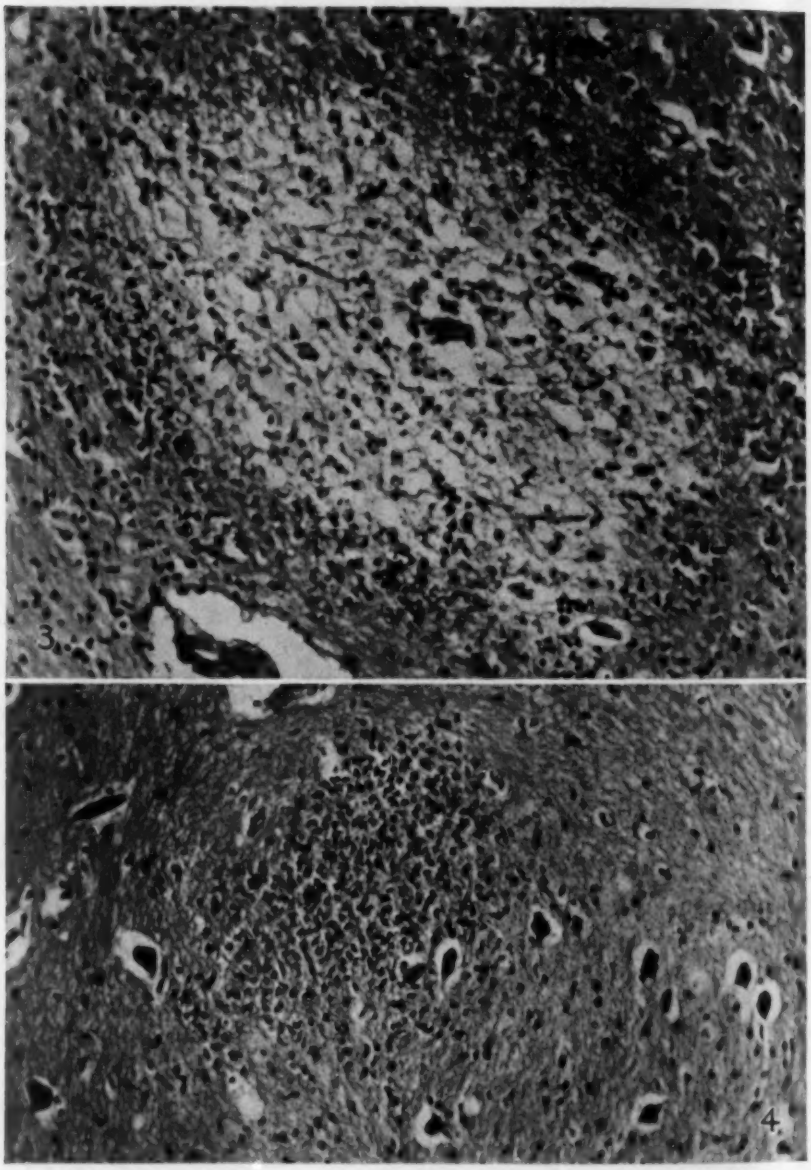

Fig. 3.—Japanese B encephalitis. Focal circumscribed lesions characterized by spongy transformation and complete destruction of the nervous parenchyma. Hematoxylin-eosin; × 160.

Fig. 4.—Japanese B encephalitis. Cellular "nodule" or "granuloma" the bulk of which is composed of proliferated microglial cells. Hematoxylin-eosin; × 220.

eaten away and invaded by various phagocytes (neuronophagia). In some instances the nerve cells are merely surrounded, but not invaded, by glia cells (satellitosis). Neuronal destruction is not always confined to cells of the anterior horn; quite frequently the cells of the posterior horn are damaged, though less severely than those of the anterior horn. The cells of Clarke's columns are, for the most part, spared from degeneration.

The neuronal destruction is always accompanied by a striking proliferation of microglia. Under low power magnification the entire gray matter of the spinal

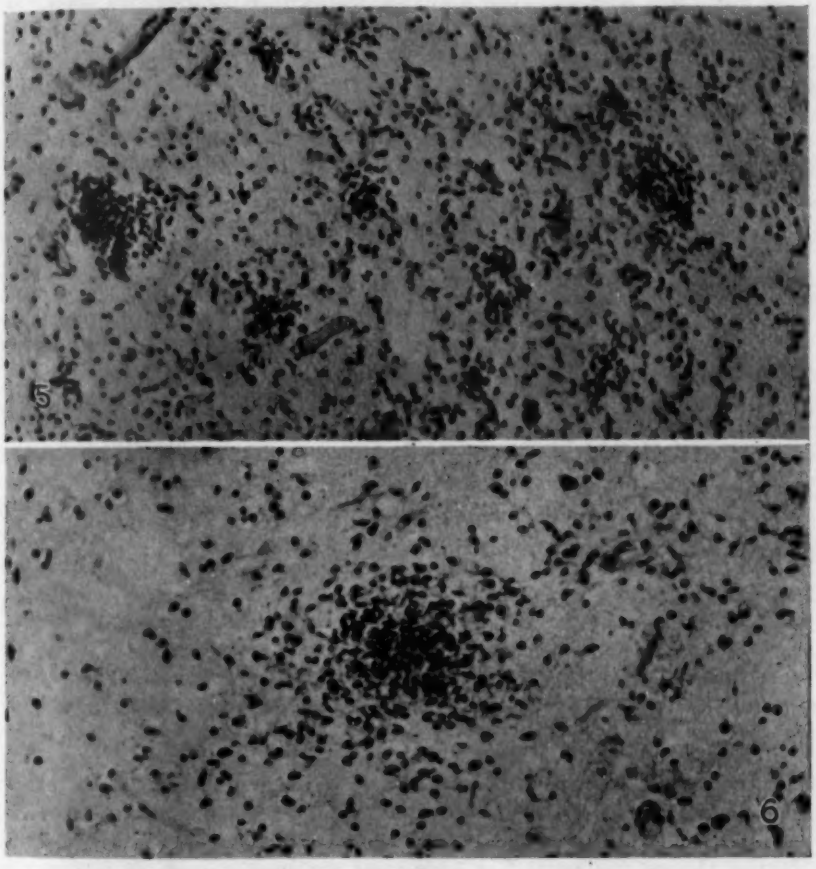

Fig. 5.—Poliomyelitis. Disseminated foci of microglial proliferation within the gray matter of the spinal cord; some of the granulomas correspond in position to severely damaged or destroyed nerve cells. Cresyl violet stain; × 160.

Fig. 6.—Poliomyelitis. Glial nodule composed of a large number of proliferated microglia cells. Cresyl violet stain; × 260.

cord manifests itself as abnormally cellular. The bulk of the cells is composed of a tremendous amount of proliferated microglia cells which, as a rule, tend to overflow the entire gray matter. Only in those areas in which the neuronal destruction is less severe do some of the microglia cells correspond in position

to severely damaged or dead nerve cells, whereas others are grouped around small veins and capillaries. In preparations stained with cresyl violet or hematoxylin and eosin, the nuclei of the microglia cells disclose considerable polymorphism and assume a rod-shaped, oval or irregular outline. The cell bodies are hardly discernible. In sections stained by the special silver carbonate method of Hortega the specifically stained microglia cells correspond in number, arrangement and distribution to those seen in preparations stained with cresyl violet.

More striking is the focal type of microglia proliferation characterized by formation of "nodules" or "granulomas" (fig. 6). The nodules are widely scattered throughout gray and white substance, and in cross sections they appear as round or ovoid bodies composed of compact accumulations of multiform microglia cells. Most of the nodules seem to be formed around severely damaged or completely destroyed nerve cells.

The bulk of mesodermal changes is seen in the smaller blood vessels (veins and capillaries) of the white matter and in the leptomeninges and is characterized by perivascular accumulation of hematogenous cells. Limited more or less to the peripheral zones of the gray matter, the infiltrating cells spread chiefly to the blood vessels running radially through the white matter and into the anterior fissure, and so reach the leptomeninges of the spinal cord. It should be emphasized, however, that perivascular infiltration often appears to be insignificant as compared with the damage sustained by the nerve cells. In areas of most severe neuronal destruction, such as the central portions of the anterior horn, there is a slight degree of perivascular cuffing. The infiltrative cells are predominantly small lymphocytes with a variable proportion of adventitial and endothelial elements. These cells are confined usually to the adventitial Virchow-Robin space. Seldom do the infiltrative cells transgress the pial-glial membrane to invade the surrounding nervous tissue. The density of the perivascular cell accumulations may vary from a few lymphocytes to a broad cuff many cells deep.

Inflammatory changes the same as those described in the spinal cord are present in the leptomeninges. Cellular exudates may encircle some of the pial vessels. The pia mater of the anterior median fissure and the entry zones of dorsal nerve roots usually are favorite localizations for the infiltrates, the composition of which is the same as that in the spinal cord. Lymphocytes, fibroblasts and endothelial cells are present in varying quantities. Plasma cells are not seen. It is worth emphasis that the degree of meningeal infiltration varies considerably at different levels of the spinal cord. The cerebral meninges are also affected, especially around the midbrain and the cerebellum.

Parallel with destruction of nerve cells and reactive glial changes, far advanced degeneration of the anterior roots is disclosed. There is destruction of the myelin sheath, which appears to be broken up into small fragments. Likewise noted is degeneration of the axons in some of the anterior roots, as well as in the white fibers of the anterior horns. In some cases the anterior roots show scattered areas of focal lymphocytic infiltration. Mesodermal alterations, as well as ectodermal, are most pronounced in the cervical and lumbar enlargements, but no level escapes severe damage.

Medulla and Pons.—The leptomeninges show pronounced lymphocytic infiltration, especially over the lateral aspects of the medulla and in the immediate vicinity of the fourth ventricle. Engorgement with hematogenous cells is more pronounced in the adventitial sheaths in the medulla and the pons than in the cord. A particularly heavy concentration of perivascular cuffing is seen in the substantia reticularis, in the region of the nucleus ambiguus, in the dorsal motor

nucleus of the vagus nerve and in the floor of the fourth ventricle. The neuronal destruction and microglial proliferation, however, are less pronounced and less severe than in the cord. Actual necrosis of nerve cells outside the region of the nucleus ambiguus and the dorsal motor nucleus of the vagus nerve is seldom seen.

The region of the olivary bodies and of the pyramidal tract completely escapes alteration.

It is noteworthy that in almost all cases examined there are numerous foci of lymphocytes in some of the cranial nerves and especially along the vagus nerve.

Midbrain.—Lesions consisting of dense perivascular infiltration are more intense in the periaqueductal region and in the substantia nigra than at any other level. As a rule, there is little actual destruction of nerve cells; complete chromatolysis, with loss of stainability of the cytoplasm, is usually the most advanced lesion encountered. There are a few disseminated foci of miliary granulomas composed of microglia cells. The leptomeninges, especially those of the interpeduncular region, show dense lymphocytic infiltration.

Basal Ganglions.—Involvement of the hypothalamic nuclei, the corpus striatum and the globus pallidus is only patchy, irregular, and moderate in degree. There are a few foci of perivascular cuffing, more marked in the paraventricular region. Neuronal destruction is not encountered. There are a few disseminated areas of focal accumulation of microglia cells.

Cortex.—No alterations of the cortical gray matter are disclosed. The leptomeninges are normal in appearance.

Cerebellum.—The dentate nucleus, the nucleus fastigii, the tegmental nucleus and the white matter often show lesions consisting of focal perivascular infiltration and focal accumulation of microglia cells. There is no evidence of severe neuronal destruction. In some cases, however, there were focal areas of neuronal damage within the cerebellar gray matter associated with microglial proliferation; these changes are similar to those seen in Japanese B encephalitis.

VIENNA, OR TYPE A, EPIDEMIC ENCEPHALITIS

The microscopic changes observed in epidemic encephalitis (lethargic encephalitis of von Economo) are somewhat similar to those in human poliomyelitis. They combine mesodermal reactions with parenchymal lesions.

In lethargic encephalitis the brunt of the pathologic process falls on the brain stem and the basal ganglions. The leptomeninges, the cerebral cortex, the subcortical white matter and the spinal cord may only occasionally disclose mild inflammatory changes. As a rule, these structures are spared from lesions. In most cases there is a striking affinity between the invading virus and the region of the substantia nigra. Even in the early stage of the disease the pigmented cells of the substantia nigra undergo changes, ranging from the milder types (chromatolysis, pallor and swelling) to complete disintegration and final disappearance. Some of the diseased nerve cells are surrounded by oligodendroglia and microglia, the components of which finally invade the cell cytoplasm (neuronophagia). Numerous amorphous granules of melanin, originating from the destroyed pigmented nerve cells of the substantia nigra, lie free in the tissue.

These neuronal alterations are frequently associated with reactive glial changes characterized by proliferation of microglia cells and cytoplasmic glia. The proliferated glia cells occasionally form "nodules" similar to those in poliomyelitis. Neuronal alterations of similar nature are seen in the pallidum of the thalamus and in the tegmentum of the pons. The tegmental nucleus and the corpus striatum usually are spared.

Essential mesodermal reactions consist in perivascular cuffing of the smaller blood vessels, varying in degree and distribution. The infiltrating cells are chiefly lymphocytes; plasma cells and polymorphonuclear leukocytes are rare. Occasionally there are fibroblasts and microglia cells. All these cells are confined to the adventitial Virchow-Robin spaces. The perivascular infiltration may be mild in degree and composed of a single ring of lymphocytes; in some cases, however, it is dense and flows over into adjoining tissue. These mesodermal changes are especially prominent in the substantia nigra, in the periaqueductal region, in the hypothalamic nuclei and in the vicinity of the third ventricle. A mild degree of perivascular cuffing may occasionally be seen in the leptomeninges, the cortex and the cerebellum.

GENERAL CONSIDERATIONS

A number of authors have described the pathologic process of Japanese, or type B, encephalitis.[3] No one, however, has directed attention to the outstanding role played by microglial proliferation in the formation of the glial nodules. All workers stress the perivascular cuffing with lymphocytes and the focal mononuclear cell infiltrations that occur in the meninges and the neuronal alterations in the cerebral cortex, the basal ganglions, the substantia nigra, the pons and the medulla. Zimmerman,[1] Haymaker and Sabin[3c] have called particular attention to striking cerebellar changes characterized by focal destruction of Purkinje cells, associated with glial proliferation. Zimmerman[1] described focal lesions resembling miliary abscesses and patches of encephalomalacia. In cases in which the disease had become chronic, the larger zones of encephalomalacia persisted as cystlike lesions. In addition, there were focal and diffuse deposits of calcium salts. Haymaker and Sabin outlined in great detail the areas of distribution of focal and diffuse lesions in a case of Japanese B encephalitis. The structures more severely affected in their case were the thalamus, the substantia nigra, the nucleus basalis, the anterior horns of the spinal cord, the cerebral cortex and the cerebellum. The nodule formation described as "neuronophagic nodules" was regarded by Haymaker and Sabin as being composed of "hematogenous and adventitial mononuclear cells." They did not deny, however, that proliferated microglia was present in some regions of the brain, notably in the cerebellum.

Among the most important problems of the pathology of infantile paralysis are (1) the localization of the disease and its distribution, (2) the predominant type of essential lesions and (3) the much disputed question of whether there is correlation between the neuronal degeneration and the inflammatory reaction.

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2. Footnote deleted.

3. (*a*) Bertrand, I., and Miyashita, K.: Rev. neurol. **65**:81, 1936. (*b*) Pette, H.: München. med. Wchnschr. **85**:1137, 1938. (*c*) Haymaker, W., and Sabin, A. B.: Arch. Neurol. & Psychiat. **57**:673, 1947. (*d*) Lewis, L.; Taylor, H. G.; Sorem, M. B.; Nocross, J. W., and Kindsvatter, V. H.: ibid. **57**:430, 1947. (*e*) Zimmerman.[1]

Until recently the attention of clinicians and pathologists was centered on the spinal cord and the medulla in cases of poliomyelitis. Histopathologic observations, as recently demonstrated in a series of cases of human poliomyelitis (Scheinker [4]), clearly indicate that no part of the central nervous system entirely escapes damage. Medulla, pons, midbrain and cerebellum are involved to a great extent in every case. Severe inflammatory lesions are especially pronounced in and around the motor nuclei of the medulla (nucleus ambiguus), in the substantia nigra and in the periaqueductal region.

In all cases examined there was striking proliferation of microglia concomitant with neuronal destruction. It should be emphasized that Hurst [5] was the first to call attention to the significant role played by microglial proliferation in cases of experimental poliomyelitis. My own recent study of lesions of the central nervous system in cases of human poliomyelitis fully corroborates the observations made by Hurst in animals.

The same study disclosed the absence of a direct parallelism between neuronal destruction and inflammatory reaction. Whereas the inflammatory changes were most pronounced in the midbrain and the medulla, in these regions the destruction of nerve cells was relatively slight and insignificant. On the other hand, in the anterior horns of the spinal cord, where destruction of nerve cells was almost complete, the inflammatory process was of only a moderate degree. It might be assumed, therefore, that neuronal degeneration is a primary process and may occur in certain localities of the nervous system in the absence of any trace of inflammation (Scheinker [6]).

Despite similarity as to essential pathologic features among cases of Japanese B encephalitis, poliomyelitis and lethargic encephalitis, their differentiation does not seem to be difficult and is to be based chiefly on the distribution of the pathologic process. Whereas in cases of Japanese B encephalitis the cerebral and the cerebellar cortex show widespread involvement, these areas are often spared in cases of poliomyelitis and of lethargic encephalitis. On the other hand, whereas in cases of human poliomyelitis the brunt of the lesions is borne by the nerve cells of the gray matter of the spinal cord and the medulla, in cases of Japanese B and of lethargic encephalitis, either no lesions occur within the spinal cord and the medulla or they are discrete.

4. Scheinker, I. M.: Histopathology in Its Clinicopathologic Aspects, Springfield, Ill., Charles C Thomas, Publisher, 1947.

5. Hurst, E. W.: J. Path. & Bact. **32**:457, 1929.

6. Scheinker, I. M.: Arch. Neurol. & Psychiat. **57**:565, 1947.

SUMMARY AND CONCLUSIONS

A study of cases of Japanese, or type B, epidemic encephalitis, poliomyelitis and Vienna, or type A, epidemic encephalitis (von Economo's lethargic encephalitis) discloses the following characteristic histopathologic changes, common to various types of virus encephalitis:

1. The morbid process is characterized by primary involvement of nerve cells of the gray matter of the brain and of the spinal cord, with relative sparing of the white substance.
2. The destructive alterations of the nerve cells are predominantly focal in character and are accompanied by focal proliferation of microglia cells, with formation of characteristic glial nodules.
3. The distribution and the severity of the inflammatory changes do not correspond with the distribution and the degree of the neuronal damage. There is no direct parallelism between the degenerative and the inflammatory lesions.
4. The differentiation among various types of virus encephalitis, from the histopathologic point of view, must be based on the distribution of the lesions. In cases of Japanese B encephalitis the structures most severely affected are the cerebral cortex, the thalamus and the substantia nigra. The lesions of the cerebellum, the medulla and the spinal cord are discrete and inconstant.

In human poliomyelitis the brunt of the morbid process is borne by the nerve cells of the gray matter of the spinal cord and of some of the cranial nerve nuclei of the medulla and the midbrain. The lesions of the pons, the cerebellum and the basal ganglions are discrete and not constant. The cerebral cortex is often spared.

The pathologic process produced in lethargic encephalitis is most intense throughout the basal ganglions and the substantia nigra. Whereas discrete focal lesions may be observed within the midbrain and the pons, they are, as a rule, absent from the cerebral cortex, the cerebellum and the spinal cord.

PATHOLOGIC CHANGES PRODUCED BY MONOCHLOROMONOBROMOMETHANE

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MONOCHLOROMONOBROMOMETHANE (CH_2ClBr) has aroused some interest recently as a potential substitute for the carbon tetrachloride used in fire extinguishers. The pure compound is a colorless, heavy liquid with a density of 1.927 at 25 C. and a distillation range of 67 to 68 C. Since no data concerning the toxicity and potential dangers of the compound were found in the literature, a study of these was undertaken by Svirbely, Highman, Alford and von Oettingen.[1] Our paper concerns the study of its pathologic effects. For this purpose, histologic studies were made of animals, chiefly mice, that died or were killed at intervals after the compound had been administered to them in an inhalation chamber or by stomach tube.

PATHOLOGIC TECHNIC

Autopsies were made on all experimental animals and unexposed controls immediately after they were killed and on many of the exposed animals that died and were not too badly autolyzed. The adrenal glands and the brain were fixed usually in Orth's solution and other organs in a solution of formaldehyde. Routine paraffin sections of the organs were stained with azure eosinate and with Van Gieson's picrofuchsin as modified by Dunn and Thompson[2] for demonstrating hemoglobin; they were also examined for hemosiderin. The patent blue stain[3] was used occasionally to confirm the presence of hemoglobin in renal casts. Frozen sections of heart, liver, kidney and adrenal gland were stained for fat

From the Pathology Laboratory and the Laboratory of Physical Biology, National Institute of Health, United States Public Health Service.

1. Svirbely, J. L.; Highman, B.; Alford, W. C., and von Oettingen, W. F.: J. Indust. Hyg. & Toxicol. **29**:382, 1947.

2. Dunn, R. C., and Thompson, E. C.: Arch. Path. **39**:49, 1945.

3. Dunn, R. C., and Thompson, E. C.: Stain Technol. **21**:65, 1946.

by the method of Lillie and Ashburn.[4] The bones were decalcified in 5 per cent aqueous formic acid as described by Lillie.[5] The heads of the mice were decalcified similarly and sectioned through the eyes and anterior portion of skull. The eyes of a few animals were enucleated and celloidin preparations were made.

ACUTE PATHOLOGIC CHANGES FOLLOWING INHALATION EXPOSURES

In most of our acute inhalation experiments we used white Swiss mice of about 20 Gm. body weight. The animals were exposed to monochloromonobromomethane for seven hours in an inhalation chamber, according to the procedure described by Werner, Mitchell, Miller and von Oettingen.[6] It was found that the $L.D._{50}$ concentration was about 12 mg. per liter or 2,300 parts per million. This was the minimal concentration of the compound that killed 50 per cent of the mice in seventy-two hours after onset of a seven hour exposure. Accordingly, to study the acute pathologic changes, several series of mice were exposed seven hours on one to five successive days to concentrations ranging from 7 to 17 mg. per liter. Several mice of each series were killed for histologic study immediately and at intervals following completion of each of the one to five exposures. Altogether, 79 mice were examined.

The chief pathologic changes observed were marked visceral congestion, fatty degeneration of the liver, the kidney and occasionally of the heart, lipoid depletion of the inner portion of the adrenal cortex, interstitial pneumonitis and opacity of one or both eyes. Less frequent lesions were tubular necrosis and hemoglobin casts in the kidney.

Fatty degeneration of the liver was perhaps the commonest and earliest lesion and was often severe even in mice that succumbed after only a few hours' exposure to as little of the compound as 7 mg. per liter. It was of the fine droplet type, usually diffuse, though often more marked in the periportal half of the lobule. It was most severe in animals that died or were killed within twenty-four hours after the end of the first exposure and was minimal or absent seventy-two hours later even in the mice that were still being exposed daily. Fatty degeneration of the kidney was not seen in mice after a single seven hour exposure to 7 mg. per liter but was moderately severe in nearly all mice dying during or shortly after a seven hour exposure to the $L.D._{50}$ concentration of 12 mg. per liter. It was minimal or absent even in mice exposed daily that died or were killed more than seventy-two hours after the end of the first exposure. One of 2 mice that died twenty-eight hours after the onset of a seven hour exposure to 17 mg. per liter showed hemoglobin casts in many scattered convoluted tubules, and a few such tubules showed necrosis of some or all of their lining epithelial cells. Slight similar changes were seen in a few other exposed mice. In the heart, slight fatty degeneration occurred focally only in occasional exposed animals.

Pneumonitis when present was not extensive and occurred particularly in mice exposed daily between twenty-four and seventy-two hours after the end of the first exposure but was infrequent thereafter. It was characterized by marked congestion and slight infiltration of the thickened interalveolar septums mainly by mononuclear cells. The lipid of the inner portion of the adrenal cortex was often depleted immediately after the end of the first exposure but was usually normal twenty-four to forty-eight hours later.

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4. Lillie, R. D., and Ashburn, L. L.: Arch. Path. **36**:432, 1943.
5. Lillie, R. D.: Am. J. Path. **20**:291, 1944.
6. Werner, H. C.; Mitchell, J. L.; Miller, J. W., and von Oettingen, W. F.: J. Indust. Hyg. & Toxicol. **25**:157, 1943.

Nearly half the mice that died during or soon after a single inhalation exposure showed a whitish opacity of one or both eyes. This opacity appeared shortly before death and usually involved the entire cornea but was often more marked at and sometimes confined to the limbus. It was not observed in mice that appeared to be recovering from an exposure.

Histologic examination of these opaque eyes was often unsatisfactory because of artefacts created in decalcifying the heads and in sectioning. The most frequent changes noted were foci of disorganization and edema in the substantia propria of the cornea with absence of the anterior epithelium or the posterior endothelium in some areas. The anterior chamber often contained some eosinophilic serous material or occasionally some polymorphonuclear and mononuclear cells.

In another experiment 47 white rats of the Sprague-Dawley strain, each weighing about 200 Gm., were killed at intervals after one to five daily seven hour exposures to concentrations of monochloromonobromomethane ranging from 7 to 20 mg. per liter. Histologic studies showed transient and infrequent slight fatty changes in the liver and the kidney. Fifteen young guinea pigs, each weighing about 300 Gm., exposed along with the rats showed changes intermediate in frequency and severity between those seen in rats and mice.

For purpose of comparison, three series of 50 mice each were exposed seven hours in an inhalation chamber to approximately $L.D._{50}$ concentrations [1] of monochloromonobromomethane (CH_2ClBr), dichloroethane (CH_2Cl_2) and carbon tetrachloride (CCl_4), respectively. In all three series of mice restlessness, muscular twitchings, uncoordinated movements, labored respiration and narcosis developed, but the mice exposed to monochloromonobromomethane recovered more slowly from the narcotic action and delayed deaths were more frequent with this than with the other compounds. The mice exposed to carbon tetrachloride showed hydropic degeneration and extensive centrolobular necrosis in the liver; this occurred within twenty-four hours after the onset of the exposure and was minimal or absent in mice killed four days later. In the kidney, fatty changes were minimal or absent during such exposure, slight to moderate twenty-four hours after the end of exposure and absent forty-eight hours after the end of exposure. Opacity of the eyes was not observed in the mice exposed to carbon tetrachloride. Nearly all the mice that died during exposure to the other two compounds showed fatty changes, severe in the liver and moderate in the kidney. Such changes were slight or absent twenty-four hours after exposure to dichloroethane but persisted for forty-eight hours in some mice after exposure to monochloromonobromomethane. Opacity of the eyes was similar in frequency and severity in mice dying from the effect of either compound.

ACUTE PATHOLOGIC CHANGES FOLLOWING ADMINISTRATION OF MONOCHLOROMONOBROMOMETHANE BY STOMACH TUBE

In these experiments a solution in olive oil (usually 50 per cent) of monochloromonobromomethane was administered by stomach tube to several series of white Swiss mice of approximately 20 Gm. body weight. The $L.D._{50}$ concentration for these mice was found to be about 4,300 mg. per kilogram.[1] Three series of mice were given a single dose of 500, 3,000 and 4,500 mg. per kilogram, respectively, and a fourth series was given 3,000 mg. per kilogram on one to ten consecutive days. Several representative mice from the three series were killed for histologic examination at intervals following the single dose, and several from the fourth series, following each of the one to ten doses. Some of the mice that died were also examined. Altogether, including control mice receiving olive oil only, 225 mice were studied in these experiments.

The acute pathologic changes were essentially similar to those following inhalation exposure to the compound as described in the foregoing section. In addition, however, some mice showed focal necrosis and hydropic degeneration of the liver; these changes occurred chiefly between twenty-four and ninety-six hours after the initial dose of the compound. A few mice showed foci of suppuration and other lesions that appeared to be incidental.

Necrosis when present almost invariably involved the anterior border and usually a small to large part of the adjacent visceral surface of the liver. Necrosis along the visceral surface occurred in a narrow, frequently discontinuous, subcapsular zone of variable width, rarely exceeding that of a lobule. The histologic picture often varied in different portions of the necrotic zone, indicating uneven development. Careful study of many such necrotic areas suggested the following chronologic development of the lesion: The earliest changes appeared to be increasing cytoplasmic oxyphilia of the liver cells, with slight pyknosis, followed by karyorrhexis, karyolysis, cellular disintegration and finally complete disappearance of the liver cells in the involved area. Meanwhile, there were apparent congestion and widening of the sinusoids in the necrotic area, with an increasing number of lymphoid and mononuclear cells and a variable number of polymorphonuclear leukocytes infiltrating this area; later the necrotic zone was replaced by a dense collection of cells with scanty cytoplasm and round to oval nuclei. This layer of cells became narrower and finally disappeared; it was rarely seen after ninety-six hours. The liver cells adjoining the subcapsular necrotic zone were generally laden with fine fat droplets; occasionally a few hydropic cells were noted, and rarely a mitotic figure.

Hydropic degeneration was less common than necrosis and occurred chiefly but not invariably in livers showing necrosis. The hydropic cells were often arranged as a narrow collar around some of the central veins. Their nuclei were often slightly pyknotic, and their cytoplasm was poorly stained and contained many vacuoles not filled with fat or glycogen.

Frequency and Severity of Changes After a Single Dose.—No significant changes were noted after administration of 500 mg. per kilogram of monochloromonobromomethane. After a single dose of 3,000 and 4,500 mg. per kilogram, fatty degeneration of the liver and the kidney was slight or absent in mice that died three or four hours later, was usually severe in the mice killed twenty-four hours later, but was generally not found in those surviving forty-eight hours or longer. Twelve of 50 mice that died or were killed within forty-eight hours showed subcapsular necrosis of the liver, and 5 showed some hydropic liver cells; slight similar changes were seen in 1 of 5 mice killed ninety-six hours after receiving the compound. Eight of 32 mice that were killed or died within twenty-four hours after administration of the compound showed hemoglobin casts in a few renal tubules; 7 of these mice received 4,500 mg. per kilogram, and 1 of the 7 showed epithelial necrosis in a few scattered convoluted renal tubules. Slight cardiac fatty degeneration was found in only 1 mouse, which died within twenty-four hours after a dose of 3,000 mg. per kilogram. Only a few adrenal glands were examined; there were indications of transient lipoid depletion of the cortex similar to that following inhalation exposure. Opacity of the eyes was not observed in the series of mice receiving a single dose of monochloromonobromomethane by stomach tube.

Frequency and Severity of Changes After Consecutive Daily Doses.—In the series of mice given multiple doses of 3,000 mg. per kilogram of monochloromonobromomethane, the lesions were somewhat more frequent, severe and prolonged than after a single dose. Fatty degeneration occurred in the liver, the

kidney and less frequently in the heart. It was most constant and severe twenty-four to forty-eight hours after the initial dose and was slight or absent after eighty hours. However, occasional mice receiving daily doses continued to show slight fatty changes, chiefly in the kidney, until seven days after the initial dose of the compound. Twenty-three of 32 mice that died or were killed within seventy-two hours after the initial dose showed subcapsular necrosis of the liver, 8 showed hydropic degeneration and 8 showed a slight to moderate increase of mononuclear cells periportally. Such changes were infrequent after three days and were not seen in mice killed more than five days after the initial dose. One mouse killed nine days after the initial dose showed a few focal and centrolobular areas of coagulation necrosis of the liver cells.

Hemoglobin casts were seen in this series in only 1 animal, which died in forty-eight hours, shortly after the third daily dose. Another mouse, which died twenty-four hours after the sixth daily dose, showed extensive tubular necrosis of the inner cortical zone of the kidney. Slight pneumonitis was found in nearly all groups but was relatively more common in mice that died or were killed between forty-eight and ninety-six hours after the initial dose.

Cloudiness of the eyes was observed in only 2 of 11 mice that died six hours after the second dose and in 3 of 8 mice that died shortly after the third daily dose of 3,000 mg. per kilogram of monochloromonobromomethane. It was found in 2 of 8 mice that died after a second daily dose of 4,500 mg. per kilogram. The histologic changes were essentially similar to those occurring after inhalation exposure.

PATHOLOGIC CHANGES AFTER MANY INHALATION EXPOSURES

To study the pathologic changes produced by prolonged exposure to monochloromonobromomethane, 19 rats, 3 rabbits and 2 dogs were killed after sixty-seven daily seven hour exposures to about 5 mg. per liter or 1,000 parts per million of monochloromonobromomethane. Usually five exposures were given weekly. No significant changes were seen except for a slight increase of hemosiderin in the spleens of the dogs and the rats and some hemosiderin deposits and an increase in fat in the kidneys of the dogs. No evidence of cirrhosis of the liver was seen.

Observations on Possible Induction of Hepatoma.—As is well known, carbon tetrachloride can induce hepatoma[7] in susceptible strains of mice, such as strains A and C3H, after prolonged administration. We have found that another halogenated hydrocarbon, 1,2-dichloropropane, can produce a similar type of hepatoma after repeated inhalation exposures. It was interesting to determine whether or not monochloromonobromomethane possessed similar potentialities. Accordingly, 100 strain A mice were given daily exposures to 1,000 parts per million of monochloromonobromomethane. The exposures were begun when the mice were 2 months of age and were given five times weekly with occasional long rest periods when these were indicated by the mortality rate and the general condition of the animals. The survivors received a total of sixty-four exposures of three to seven hours each in a period of about five months. Another 45 mice of the C3H strain were first exposed at 3 to 7 months of age to similar concentrations of the compound. The survivors received a total of forty-nine such exposures in a period of four months.

Most of the mice died at irregular intervals during the period of exposure; some died or were killed for histologic study at intervals following this period

7. Edwards, J. E.: J. Nat. Cancer Inst. **2**:197, 1941.

of exposure, and the remainder, totaling 21 mice (1 strain A and 20 C3H), were killed at 13 to 16 months of age. The animals that were killed and some that died were studied histologically. The mice that died during the exposures generally showed slight fatty changes in the liver and the kidney. Two strain A mice that died during the fourth daily exposure showed extensive tubular necrosis of the inner zone of the renal cortex. Several others that died during exposure showed coagulation or karyorrhectic necrosis of a few isolated liver cells. Others that died or were killed showed pneumonitis, pulmonary adenoma, renal abscess and other lesions that appeared to be incidental and not directly related to the exposures. None showed any evidence of induced hepatoma similar to that produced by carbon tetrachloride.

COMMENT

The transient character of the lesions found in most of the animals indicates that most animals are capable of building up tolerance to intermittent exposures of monochloromonobromomethane. The occurrence of occasional deaths among the mice receiving inhalation exposures over a long period and the occurrence of slight fatty changes in the viscera of such animals indicate that this tolerance is not absolute. Perhaps the loss of tolerance to monochloromonobromomethane is due to gradual accumulation of bromide in the tissues.[1] After a rest period such mice were often able to tolerate daily exposures for some time.

The histologic changes in the cornea are not unlike those described as following exposure to dichloroethane.[6] However, the cloudiness of the eyes differs from that produced by dichloroethane in several respects. Heppel, Neal, Endicott and Porterfield[8] found that only in dogs and foxes did bilateral corneal turbidity develop after inhalation exposure to dichloroethane, that such animals often remained in good health and that the process was reversible. In the present experiments with monochloromonobromomethane we have not observed such changes in dogs; we have observed either unilateral or bilateral cloudiness of the eyes in some of the exposed mice only, generally occurring shortly before their death. Such changes may ensue after the animals have been exposed to monochloromonobromomethane either by inhalation or, less frequently, by oral administration. The latter observation indicates that contact between the cornea and the vapor is not necessary. Chemical analysis of several enucleated eyes revealed a high concentration of bromide, further indicating that the eye may be injured by the absorbed compound. The greater frequency and the earlier onset of opacity following inhalation exposure suggests, however, that contact of the vapor with the outer surface of the cornea is an important factor in producing the opacity in mice receiving inhalation exposures.

8. Heppel, L. A.; Neal, P. A.; Endicott, K. M., and Porterfield, V. T.: Arch. Ophth. **32**:391, 1944.

According to Cogan and Kinsey,[9] the epithelium and the endothelium of the cornea are semipermeable with respect to sodium chloride. Injury of these membranes results in a disturbance of osmotic forces resulting in swelling and opacity of the cornea. For the cornea to remain transparent, it must be maintained in a deturgescent state. Heppel, Neal, Endicott and Porterfield [8] stated that the basis of the lesion produced by dichloroethane is believed to be injury to the posterior or endothelial surface of the cornea. Further work is necessary to determine whether or not the cloudiness of the eyes produced by monochloromonobromomethane and dichloromethane is due to a similar injury to the cornea and whether or not such injury is caused by a specific action of these compounds and is limited to mice.

The fact that necrosis and hydropic degeneration of the liver occurred only in mice receiving the compound by stomach tube is interesting. It suggests the possibility that absorption of the compound from the intestinal tract and its conveyance to the liver by the portal vein render it relatively more injurious than when it is conveyed to the liver by the systemic circulation after pulmonary absorption. Perhaps it reaches the liver in greater concentration and acts over a longer period per day, since it may be gradually absorbed from the intestinal tract during a period exceeding the seven hours of the inhalation exposures. The occurrence of the necrosis along the visceral surface of the liver deserves further study.

SUMMARY

The chief pathologic changes observed in mice following inhalation exposure to monochloromonobromomethane were marked visceral congestion, fatty degeneration of the liver, of the kidney and occasionally of the heart, lipoid depletion of the adrenal cortex, interstitial pneumonitis and, in animals that died, opacity of the eyes. Such changes were minimal or absent even in mice given several consecutive daily exposures if they were killed more than seventy-two hours after the end of their first exposure. Mice exposed to dichloroethane showed similar changes but recovered more rapidly. Necrosis and cirrhosis of the liver similar to that following carbon tetrachloride was not observed in mice, rats, rabbits and dogs even after many inhalation exposures.

Mice receiving daily doses of monochloromonobromomethane in olive oil by stomach tube showed changes similar to those following inhalation exposure. However, opacity of the eyes was relatively uncommon, and hydropic degeneration and subcapsular necrosis of the liver occurred frequently between twenty-four and seventy-two hours after the initial dose of the compound.

9. Cogan, D. G., and Kinsey, V. E.: Arch. Ophth. **28**:661, 1942.

EXPERIMENTAL CORONARY SCLEROSIS

I. Medial Degeneration as the Primary Lesion in Coronary Sclerosis of Cockerels

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THE OBSERVATIONS reported here were made in the course of a series of long term studies of experimental coronary sclerosis in chickens. Our studies had several objectives, most of them being concerned with the final stages of the disease in middle-aged and elderly fowl. These final stages have not yet been reached; but during routine examinations of fowl in the earlier parts of the experiments a medial lesion has been observed which seems to be of prime importance in the genesis of coronary sclerosis in chickens. The present report deals exclusively with this lesion as observed in White Leghorn and Barred Rock cockerels. Detailed reports on other aspects of the investigations will be made at a later date.

MATERIAL AND METHOD

White Leghorn cockerels were used in the first experiment. Two hundred and twenty-five 1 day old male chicks were obtained from a commercial hatchery and fed a regular starting ration [1] until they were 14 weeks old. The colony was then divided into two groups, and the birds in each were fed a regular growing

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1. The starting ration consisted of the following ingredients, in percentages: ground yellow corn, 20.0; ground wheat, 10.0; wheat bran, 10.0; wheat shorts, 10.0; rolled oat groats, 10.0; ground whole oats, 8.0; ground barley, 5.0; dehydrated alfalfa, 3.0; dehydrated cereal grass, 1.0; buttermilk powder, 5.0; fish meal, 3.0; meat meal, 5.0; soybean oil meal, 6.5; oyster shell (fine), 1.75; grit (insoluble), 1.0; salt (containing 0.02 per cent potassium iodide), 0.5; fortified fish liver oil (containing 400 AOAC [i. e., Association of Official Agricultural Chemists] chick units of vitamin D, and 3,000 U.S.P. [or international] units of vitamin A per gram) 0.25.

ration.[2] The birds of group 1, 125 in number, were fed the growing ration, to which 2 per cent dry commercial cholesterol (The Armour Laboratories) was added and intimately admixed. The birds of group 2, 100 in number, were fed the growing ration only. The two groups of cockerels were housed in adjacent pens of the same size, exposure and ventilation. The birds of group 1 were maintained on the cholesterol-containing diet for eleven months, and at the end of this time they were fed the same mash that was fed to the birds of group 2.

The weight of each bird and the feed consumption of each group were noted at regular intervals. Blood cholesterol levels were determined each month on pooled blood samples and on individual specimens, a modified Schoenheimer-Sperry technic being used. A detailed report of the biochemical technics and findings will be given in a later paper.

Representative birds were taken from each group and killed at regular intervals after the start of cholesterol feeding, viz., 3 birds at three and a half months, 10 birds at from five to six months, 10 birds at nine months and 10 birds at twelve months. Examinations have therefore been carried out on 33 birds from each group to date.

The technic of the pathologic examination of each specimen was as follows: The aorta was opened and examined for gross evidence of atherosclerosis. If plaques were noted, a single block was taken through the apex of the most advanced lesion. If no plaques were evident, a block of tissue was taken through the ascending aorta and another in the midabdominal region. The heart and the aorta were then placed in 4 per cent formaldehyde solution, and after fixation the heart (and its coronary arteries) was treated in a special fashion. Approximately the upper three fifths of the organ was divided into four blocks, each block being cut horizontally through the entire thickness of the myocardium. The first block was taken at the level of the atrioventricular groove, and the other three, at lower levels. The blocks of tissue were embedded in paraffin, and serial sections, at intervals of 400 microns, cut through the entire thickness of each block. In this manner, from 20 to 30 equidistant sections, each of which contained three cross sections of the main coronary arteries, were obtained from each specimen. The sections were stained routinely with hematoxylin and eosin, although Weigert's elastic tissue stain was used on a few. An occasional frozen section of a special part of a coronary artery was stained with Herxheimer's scarlet red for fat.

A second experiment was carried out with 84 Barred Rock cockerels. The technic was similar to that in the first experiment except that the birds, in groups of 12, were fed either the growing ration only, the growing ration plus 2 per cent dry cholesterol, the growing ration plus cholesterol plus cottonseed oil in various concentrations, or the growing ration plus cholesterol plus 5 per cent corn oil. A detailed description of the feeding technic used in this experi-

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2. The growing ration consisted of the following ingredients, in percentages: ground yellow corn, 20.0; ground wheat, 10.0; wheat bran, 10.0; wheat shorts, 10.0; rolled whole oats, 20.75; ground barley, 15.0; dehydrated alfalfa, 3.0; dehydrated cereal grass, 1.0; buttermilk powder, 2.0; fish meal, 2.0; meat meal, 1.5; soybean oil meal, 2.0; butyl fermentation solubles, 0.25; oyster shell (fine), 1.5; steamed bone meal, 0.25; salt (containing 0.02 per cent potassium iodide), 0.5; fortified fish liver oil (containing 400 AOAC chick units of vitamin D, and 3,000 U.S.P. [or international] units of vitamin A per gram), 0.25.

To these mixtures 0.0125 Gm. of manganese sulfate (anhydrous, technical grade) was added for every 100 Gm. of ration.

ment, as well as an account of the effects of the various diets on the blood cholesterol levels and on the incidence of coronary sclerosis, will be given in a later publication.

OBSERVATIONS

The results of the first experiment, in which a group of White Leghorn cockerels were fed dry cholesterol for eleven months, indicate that this feeding technic has certain definite advantages over cholesterol–cottonseed oil mixtures as used by Dauber and Katz [3] for the production of end stage coronary sclerosis in chickens. The dry cholesterol was well tolerated by the birds for most of the experimental period. They showed a normal gain in weight, and the mortality was no greater than that in the controls. After six months of cholesterol feeding, the blood cholesterol levels were approximately four times those of the control birds (table 1). A significant degree of coronary sclerosis occurred in the treated cockerels during the feeding period: At least

TABLE 1.—*Effect of 2 Per Cent Dry Cholesterol Feeding on the Blood Cholesterol Levels of Cockerels*

Duration of Cholesterol Feeding	Total Blood Cholesterol Levels, Mg./100 Cc.	
	Control Birds	Treated Birds
2 months	108	275
3 months	125	400
4 months	106	388
5 months	102	487
8 months	94	325

Note: Each of the determinations represents the level of total blood cholesterol in a pooled blood sample from 20 birds.

one atherosclerotic plaque comparable to that shown in figure 9 has been found in approximately half of the cholesterol-fed birds examined to date. A more detailed account of these attempts to produce end stage coronary sclerosis in chickens by dry cholesterol feeding will be given at a later date.

Of most interest in the White Leghorn cockerels were the findings (1) that the incidence of coronary sclerosis was approximately the same in the controls as in the cholesterol-fed birds; (2) that cholesterol feeding accelerated the sclerotic process in individual plaques, and (3) that a degenerative medial lesion preceded the intimal changes in the treated and the control cockerels. These three findings will be discussed in detail in the present report.

Definite evidence of spontaneous arteriosclerosis has been found in the coronary arteries of 25 of the 33 control White Leghorn cockerels examined to date. Lesions similar to that illustrated in figure 3 have been noted in approximately one third of the 865 individual sections of

3. Dauber, D. V., and Katz, L. N.: Arch. Path. **34**:937, 1942; **36**:473, 1943.

the 33 hearts from this group. All three coats of the arterial wall were involved in the disease process, but the medial changes were the striking ones. Here, distention of cells with material that did not take the stain, pyknotic nuclei, fragmentation of elastic tissue and round cell infiltration were the prominent features (figure 4). The appearance of the medial lesion, on the whole, was that of hydropic degeneration. It differed

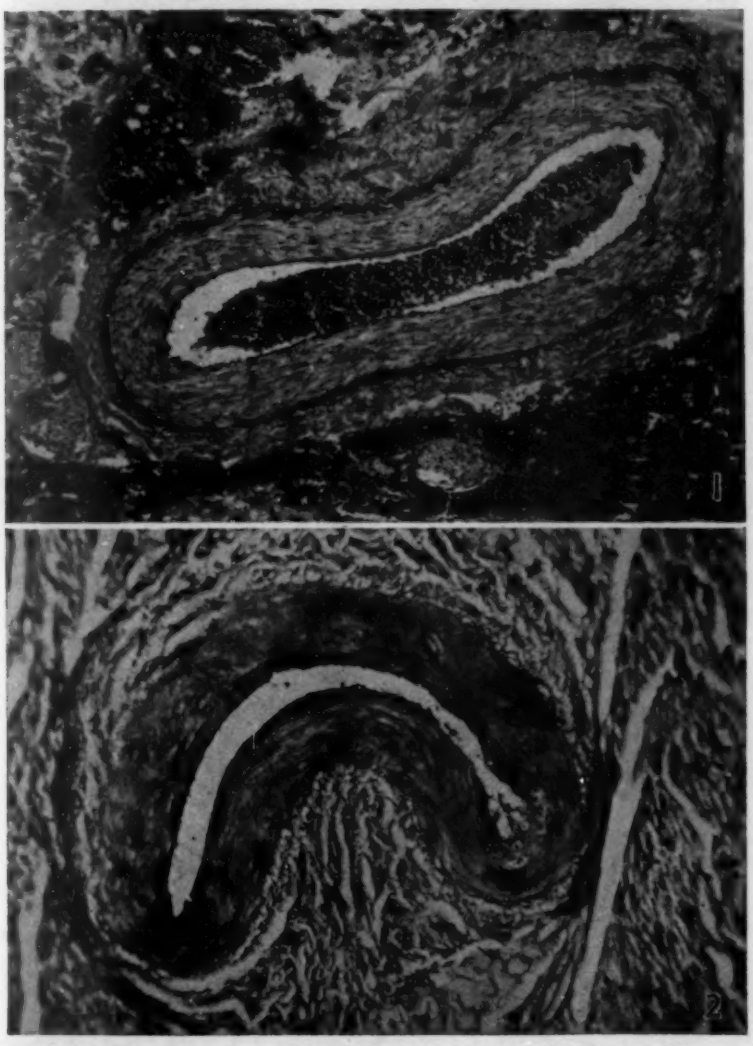

Fig. 1.—Photomicrograph of a normal coronary artery of a White Leghorn cockerel. Hematoxylin and eosin stain; × 100.

Fig. 2.—Photomicrograph of an early spontaneous lesion of a coronary artery of a control cockerel. At the right hand extremity is a focus of hydropic degeneration of the inner layers of the media. The adjacent endothelium shows evidence of early proliferation. Hematoxylin and eosin stain; × 100.

from the spontaneous medial lesions described in other species in that there was a complete lack of calcification. The intima and the adventitia adjacent to the focus of medial disease were usually affected. The internal elastic lamina was duplicated and often disrupted. The intima showed slight but definite changes in most instances. Proliferation of

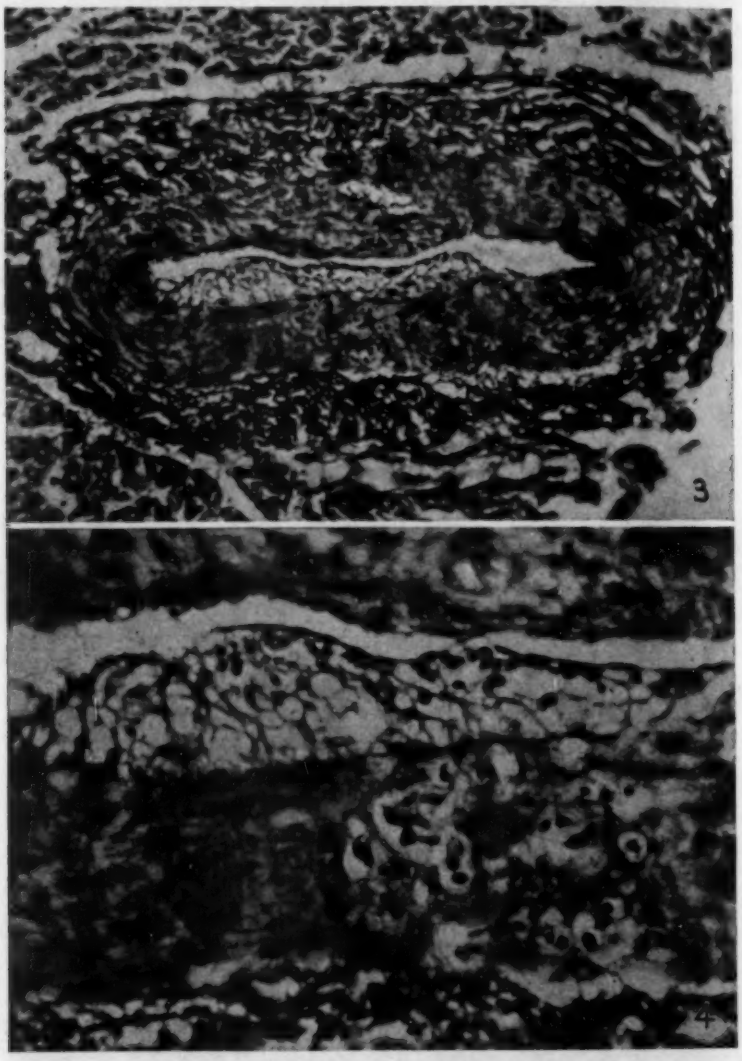

Fig. 3.—Photomicrograph of a coronary artery of a control cockerel showing a typical lesion of spontaneous arteriosclerosis. An intimal plaque is shown, and beneath it is a large focus of hydropic degeneration. Stainable fat has not been demonstrated in lesions of this type. Hematoxylin and eosin stain; × 150.

Fig. 4.—A high power photomicrograph of the arterial lesion shown in figure 3. The "hydropic" nature of the medial lesion is clearly demonstrated. Hematoxylin and eosin stain; × 450.

endothelial cells, which were usually vacuolated, resulted in the formation of small plaques. In only 2 of the 33 specimens examined did an individual plaque exceed in size that illustrated in figure 3. Fibroblasts have been found in the thickened intima, but stainable fat has not been demonstrated either in the plaques or in the underlying foci of medial disease. A localized round cell infiltration of the adventitia was usually found at any point where the adjacent media was affected.

Serial sections have shown that the primary site of spontaneous arterial disease lay in the media. A White Leghorn cockerel (a control bird from an auxiliary experiment) was selected because its coronary arteries showed large numbers of medial degenerative foci but only slight associated intimal changes. A fifth block of the myocardium, closer to the apex of the heart than the four blocks that were examined routinely, was embedded in paraffin and sectioned serially at intervals of 5 microns. Every section, except those that were accidentally lost, was stained and examined. Four fairly complete series of sections of individual arterial lesions were obtained from the specimen. In three of the series both media and intima were involved in the degenerative process; but the fourth series definitely localized the site of disease in the outer coats of the vessel. A diagrammatic reconstruction of the lesion is shown in figure 5, and in this the following points will be noted: The lining endothelium is swollen but not proliferating; the internal elastic lamina is duplicated, and the media shows advanced hydropic degeneration, disruption of muscle and elastic fibers, and round cell infiltration. It will also be noted in the diagram that a single section was accidentally lost from either extremity of the medial lesion; but it is felt that this loss does not nullify the claim that the intima, for all practical purposes, was free from disease at the point in question.

The feeding of dry cholesterol to the White Leghorn cockerels of group 1 accelerated, quite definitely, the arteriosclerotic process in the coronary arteries; but the incidence of the disease was not altered significantly. As shown in table 2, the total number of spontaneous intimal and medial lesions in the control birds was approximately the same as that in the cholesterol-fed birds. Cholesterol feeding, however, altered markedly the size and the structure of the arterial lesions. The intimal plaques, instead of being small and insignificant (like the typical spontaneous lesion shown in figure 3), often became quite large and encroached on the arterial lumen. In approximately half of the cholesterol-fed birds examined, one or more intimal plaques comparable in size to that shown in figure 9 have been found in some part of the coronary system. Lipoid material, readily stained with fat stains, was a striking constituent of the intimal lesions. In most cases it was present in foam cells (figures 7 and 8), and in the more advanced lesions the deposition of lipoid material was accompanied by some fibroblastic

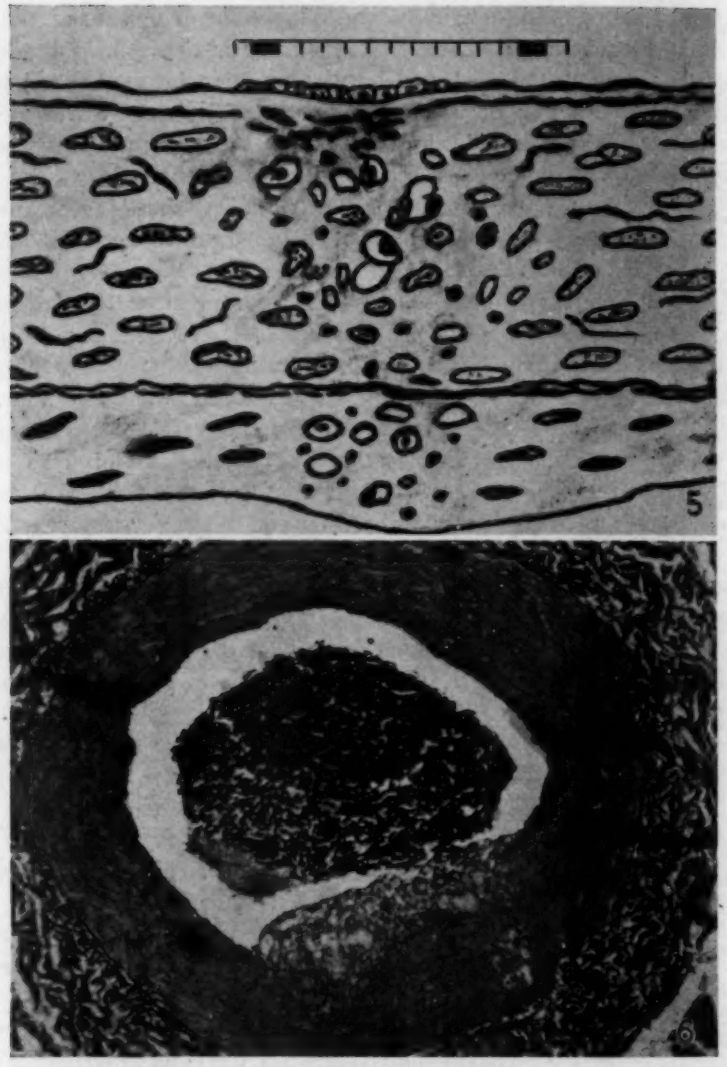

Fig. 5.—Diagrammatic reconstruction of the microscopic picture noted in 15 consecutive sections cut through a medial lesion of an artery of a control cockerel. The endothelium is swollen but is still a single layer in thickness; the internal elastic lamina is duplicated, and the media and the adventitia are occupied at one point by a large focus of hydropic degeneration. The scale below the diagram shows the location of each section. The two shaded areas indicate where sections were accidentally lost.

Fig. 6.—Photomicrograph of an early arterial lesion in a cholesterol-fed cockerel. The changes are similar to those in the spontaneous lesion shown in figure 3, but a few foam cells are present, and stainable fat has been demonstrated in lesions of this type. Hematoxylin and eosin stain; × 100.

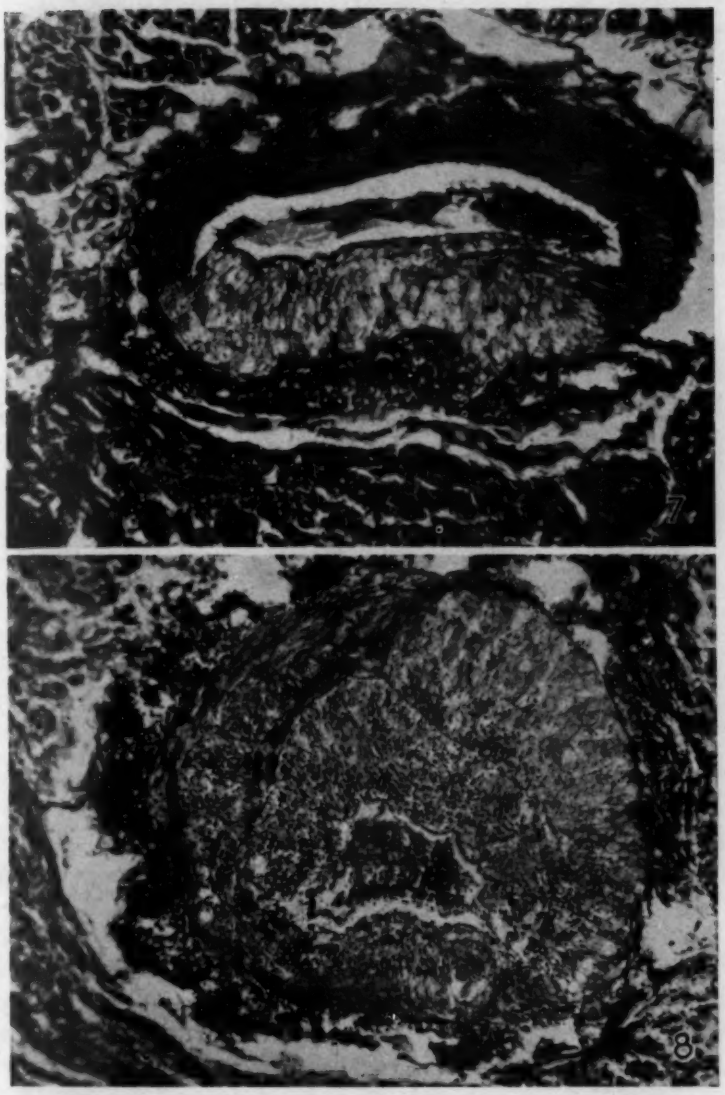

Fig. 7.—Photomicrograph of an arterial lesion of moderate severity in a cholesterol-fed bird. The internal elastic lamina is disrupted, and the intimal plaque and the adjacent media show extensive deposition of foam cells. The sharp line of demarcation between the degenerated muscle and the normal muscle is clearly shown. Hematoxylin and eosin stain; × 100.

Fig. 8.—Photomicrograph of a point of marked arteriosclerosis in a coronary artery of a cholesterol-fed cockerel. The arterial lumen is stenosed by a massive deposit of foam cells in the intima; the media is involved in a similar process of fatty degeneration. The internal elastic lamina is intact at some points. The sharp demarcation of diseased muscle from normal muscule is again evident. Hematoxylin and eosin stain; × 100.

proliferation. We have been unable to establish any definite relationship between the size of the intimal plaques and the duration of cholesterol feeding or the degree of hypercholesteremia. All that can be stated is that with cholesterol feeding lipoid material was deposited in preexistent intimal plaques and that this deposition was accompanied by an acceleration of the arteriosclerotic process. Striking changes of the medial and adventitial lesions also resulted from cholesterol feeding. In the earlier stages of the experiment, the foci of hydropic degeneration of the media were infiltrated by varying numbers of foam cells, and often these cells were so numerous that they obscured all detail in the musculoelastic coat (figures 7 and 8). Later a good deal of free fat, crystalline material, necrotic debris and calcific deposits made their appearance (figure 9). A prominent feature of all medial lesions in the cholesterol-fed birds was their sharp demarcation from the adjacent normal tissues. Not infrequently the adventitia was involved in the atheromatous

TABLE 2.—*Incidence of Intimal and Medial Disease in Cholesterol-Fed and Control Cockerels*

	Control Birds	Cholesterol-Fed Birds
Number of birds studied	33	33
General incidence of coronary sclerosis	25 of 33	27 of 33
Total number of sections examined	865	862
Number of sections showing intimal plaques	284	325
Number of sections with intimal plaques and with associated medial lesions	256	318

process, and this accounts for the nodular outpouchings of the arterial wall seen so often in older subjects (figure 10).

The observations on White Leghorn cockerels, just described, have been confirmed in a general way in a second experiment in which Barred Rock cockerels were used. In this breed, spontaneous medial and intimal lesions of the coronary arteries have been noted repatedly, and cholesterol feeding accelerated the disease. When 20 per cent cottonseed oil was added to the cholesterol diet, the atheromatous changes in the coronary arteries (and in the aorta) were much exaggerated; but these findings and others will be discussed in more detail in a later report.

Although our studies have been concerned almost exclusively with lesions of the coronary arteries of cockerels, observations have also been made on the aorta and on certain visceral arteries. One point has been quite clear—the coronary arteries of our two breeds of cockerels have been found to be much more susceptible to spontaneous disease and to the accelerating effect of cholesterol feeding than these other vessels. Routine gross and microscopic examinations of the aorta, for example, have revealed no lesions, or insignificant ones, in birds with coronary

sclerosis of a definite degree. When aortic lesions were present, however, they were often accompanied by medial lesions indistinguishable from those seen in the coronary system. Another incidental observation

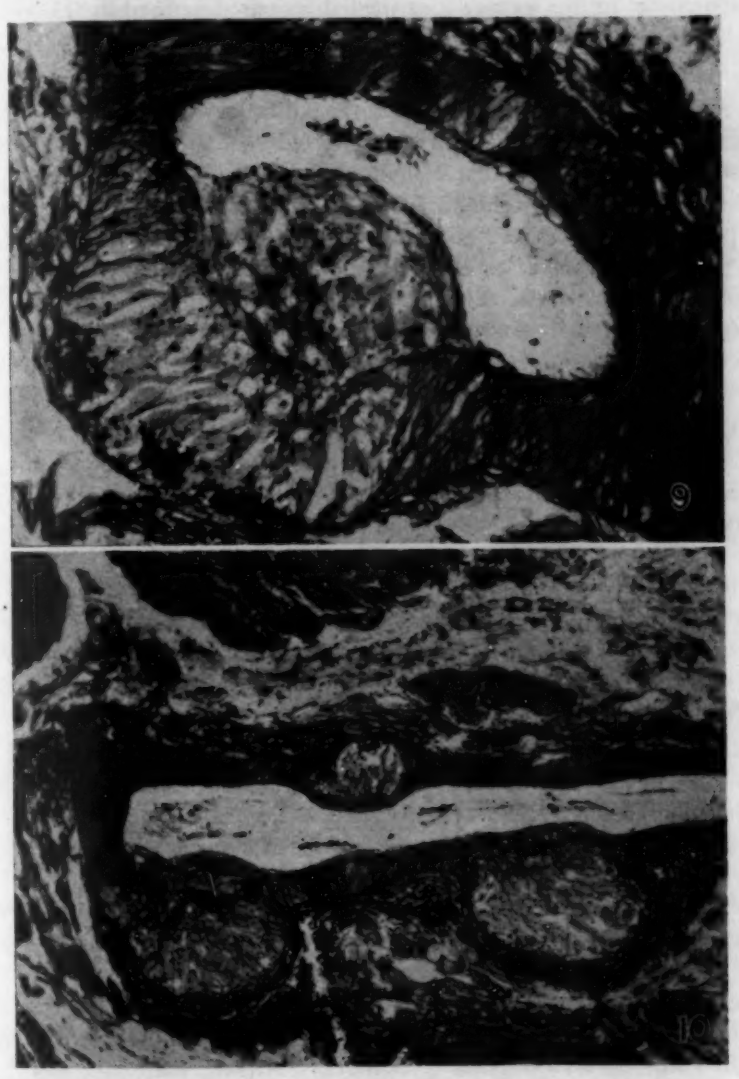

Fig. 9.—Photomicrograph of an advanced lesion of a coronary artery of a cholesterol-fed cockerel. The intimal plaque consists principally of foam cells, but the underlying media shows deposition of crystalline material and calcification. The arterial wall bulges at the point of disease. Hematoxylin and eosin stain; × 150.

Fig. 10.—A low power photomicrograph of a longitudinal section of a coronary artery of a cholesterol-fed cockerel. The focal distribution of the lesions and the outpouchings of the arterial wall at the points of disease are shown. Hematoxylin and eosin stain; × 75.

made in the course of these experiments was that foci of round cell infiltration of the myocardium were often present, especially in the White Leghorn cockerels. These "inflammatory" foci may have been related in some way to the spontaneous medial lesions described in this report.

COMMENT

Spontaneous arteriosclerosis of the aorta of the chicken has been described before. Uchiyama [4] has reported spontaneous disease of this vessel as being present almost invariably by the end of the second year of life. Dauber [5] has found spontaneous arteriosclerosis of the aorta in 45 per cent of older hens and cock birds. The findings in the birds in the present experiments indicate that spontaneous arteriosclerosis of the coronary arteries is also extremely common. Indeed, they show that the coronary arteries are more susceptible to spontaneous disease than are other vessels—a point of some practical importance to those who are studying arteriosclerosis in this species.

Contrary to the reports of others, in our control groups of cockerels the primary site of degeneration lay in the media, not in the intima. Serial sections have shown that the media was involved in a degenerative process which antedated the proliferation of intimal tissues. Dauber,[5] and Dauber and Katz,[3] in their studies on spontaneous and cholesterol-"induced" arteriosclerosis of chickens, have described involvement of the media in many of their specimens; but they interpreted this as being secondary to the intimal change. As they do not appear to have carried out serial sections of early lesions, it is quite conceivable that the disease in their birds also originated in the media.

In our cholesterol-fed birds the arterial lesions, although altered by deposition of lipoid material, must have been identical in origin with those of the spontaneous variety. If entirely new lesions had been initiated by the cholesterol feeding, there would have been clearcut examples both of hydropic and of fatty degeneration in individual birds. And if this had been the case, the total number of lesions occurring in the cholesterol-fed group would have been much greater than that in the control group. However, the incidence of disease has been found to be practically the same in the two groups (table 2). We conclude, therefore, that the feeding of cholesterol to the White Leghorn and Barred Rock cockerels resulted in purely secondary changes. Lipoid material was laid down at points of preexistent disease in the arterial wall; it accelerated the arteriosclerotic process, especially in the intima, and stenosing plaques were thus produced. In view of these findings we cannot support the claim of others that the feeding of cholesterol to birds produces

4. Uchiyama, T.: Virchows Arch. f. path. Anat. **277**:642, 1930.

5. Dauber, D. V.: Arch. Path. **38**:46, 1944.

primary lesions in arterial walls.[6] And it is to be noted, further, that Duff,[7] in cholesterol feeding experiments in rabbits, found cholesterol deposits localized in sites of previous injury.

Having eliminated excessive cholesterol intake as the cause of the coronary sclerosis in our two breeds of cockerels, other possible etiologic factors should be discussed. There is said to be a wide range of the total blood cholesterol level of normal chickens, and it has been suggested that transient hypercholesteremia of metabolic origin might be the cause of spontaneous arteriosclerosis in this species.[8] This mechanism appears to us to be improbable. Individual determinations of the levels of the total blood cholesterol of 30 of our control group of cockerels showed none to be in excess of 140 mg. per hundred cubic centimeters. And of more importance was our failure to demonstrate stainable fat in numerous sections of two of the largest spontaneous lesions in our control group of birds. It is realized, of course, that lipoid material may appear in more advanced spontaneous plaques in older cockerels, and this has been reported by Dauber.[5]

The usual causes of spontaneous medial degeneration and necrosis as reviewed by Duff [7] and by Hueper [8] could not have been responsible for the arterial disease encountered here. The administration of epinephrine, nicotine, tyramine, barium chloride, hydrastine, digitalin, diphtheria toxin and other agents produces primary medial lesions in rabbits, but none of these substances was given to our birds.

The sulfonamide compounds in repeated doses are said to cause arterial lesions in man and in experimental animals. In this connection it is to be noted that each bird in our two experiments received a single dose of a sulfonamide compound while at an early age. The White Leghorn cockerels were given sulfanilamide for one day to combat an outbreak of acute staphylococcosis. The Barred Rock cockerels received one treatment with azosulfamide as part of a prevention campaign against coccidiosis. However, if a single dose of a sulfonamide compound can cause widespread and persistent arterial disease, like that found here, it must indeed be highly deleterious in the fowl.

Bacterial or virus infections, or allergy to the products of these agents, cannot be eliminated as causes of the primary lesions in our cockerels. Chickens, on the whole, are highly susceptible to Salmonella pullorum infections, to coccidiosis, lymphomatosis of various types and various other contagious diseases. Anatomically, the medial lesions in

6. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933, chap. 10. Dauber and Katz.[5]

7. Duff, G. L.: Arch. Path. **20**:81 and 259, 1935.

8. Hueper, W. C.: Arch. Path. **38**:162, 245 and 350, 1944; **39**:51, 117 and 187, 1945.

our birds had a certain resemblance to types of infectious arteritis in man, particularly to syphilitic aortitis. The spotty distribution of the medial and intimal changes, the round cell infiltration of the adjacent adventitia, the nodular outpouchings of the arterial wall in older birds, and the frequent association of the arterial lesions with cellular infiltrations of the myocardium all suggests an inflammatory mechanism, operating perhaps through the vasa vasorum. It is of some interest to note that Dauber and Katz [3] have also observed instances of intercurrent carditis in their material.

Whether human arteriosclerosis bears any relation to the arterial lesions reported in this paper is not clear. Morphologically, there are certain obvious differences between the two diseases. The popular conception of human arteriosclerosis of the atherosclerotic type is that it is primarily and predominantly a disease of the intima. Medial involvement, when it occurs, is considered to be purely secondary. If this is true, spontaneous arteriosclerosis of chickens is a disease process quite distinct from the human type. But if it is ever shown that human atherosclerosis also originates in a focus of medial degeneration or necrosis, however transient and apparently innocuous this focus may be, then the chicken may be an invaluable experimental tool in the elucidation of the cause. In the chicken the primary medial lesions are readily apparent, even on the most cursory type of examination.

SUMMARY AND CONCLUSIONS

A high incidence of spontaneous arteriosclerosis of the coronary arteries, greater than that observed in the aorta, has been found in males of two breeds of chickens. Serial sections have shown that the primary lesion of these arteries is a focus of hydropic degeneration of the medial coat. The possible causes of the primary lesion are discussed, and it is suggested that the lesion may be infectious in origin.

The feeding of dry cholesterol to the birds in these experiments produced changes in the coronary arteries that were purely secondary. Lipoid material was laid down at points of preexistent spontaneous disease, and it accelerated the arteriosclerotic process, especially in the intima, and resulted in the formation of stenosing plaques of the atherosclerotic type.

BARBITURATE-OPIATE INTOXICATION WITH NECROSIS OF THE BASAL GANGLIONS OF THE BRAIN

Report of a Case

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THIELLS, N. Y.
AND
FRANK T. JOYCE, M.D.*
DENVER

ALTHOUGH the study of the lesions of the brain in instances of fatal poisoning with barbiturates or opiates appears of special interest in view of the selective effect of these drugs on the nervous system, only few of these cases have been the object of extensive pathologic examination. It is the purpose of this report to describe the case of a patient who, following overdosage of a barbiturate and an opiate, showed clinical evidence of diffuse involvement of the central nervous system. On postmortem examination, the main pathologic change consisted of extensive bilateral necrosis of the basal ganglions. In addition, small foci of necrosis were scattered throughout the brain. The nature of these pathologic changes will be commented on and their genesis briefly discussed.

REPORT OF CASE

J. S., a white woman aged 30 years, was admitted to Brooke General Hospital, May 26, 1944, with the diagnosis of toxic encephalopathy. The patient had been afflicted for many years with severe periodic migrainous attacks and had repeatedly used therapeutic doses of pentobarbital sodium ("nembutal") and dihydromorphinone hydrochloride ("dilaudid") without showing apparent harmful effects. On March 8, 1944 a severe attack of migraine developed. During the day she ingested "at least fifteen" 1½ grain (0.09 Gm.) capsules of the first named drug and continued to take "one capsule after another" on the following day. In addition, she gave herself hypodermically ten 1/10 grain (4 mg.) "dilaudid" tablets. It is probable that the patient had contemplated suicide at that time. It was not until March 10 that she was found unconscious in her room when her apartment was forcibly entered.

The patient was admitted to Fitzsimmons General Hospital, where she remained in coma for three weeks. During the first days she was "extremely cyanotic" and "showed evidence of depression of both circulatory and respiratory centers." After treatment, which consisted essentially of continuous administration of oxygen and repeated blood transfusions, gradually improvement was noted. On May 26 she was transferred to Brooke General Hospital. Here, examination showed

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motor quadriplegia, which was more pronounced in the lower extremities. There was contracture in flexion of both legs and arms. The muscles of the face and the neck showed continuous involuntary movements, athetoid in character. The patient's speech was difficult to understand because of marked slurring. Mentally, she appeared severely deteriorated, showed pronounced defect of memory, being unable to recall any event which had occurred after 1941, and suffered from repeated episodes of noisy excitement and forced crying.

During the following months, the condition grew progressively worse, contractures increased, decubitus and urinary incontinence developed, and on two occasions generalized convulsive seizures were observed. Mentally, she became more and more confused, restless and excitable. Since sedation was necessary to secure some amount of quietness, she received for several months from 9 to 15 grains (0.58 to 0.97 Gm.) of "seconal sodium" daily, and 1/16 grain of dihydro-

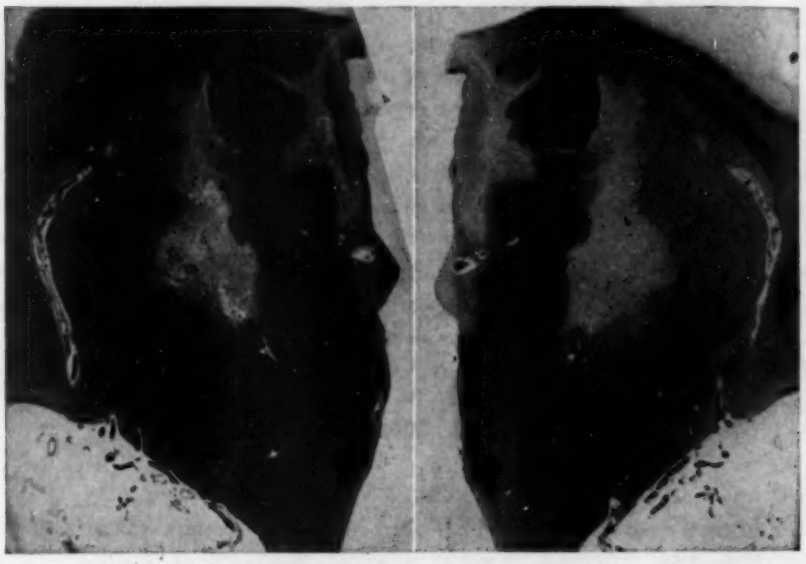

Fig. 1.—Characteristic necrosis of the basal ganglions, mainly of the globus pallidus.

morphinone hydrochloride from three to six times a day. In November 1944 death occurred, following manifestations of bronchopneumonia. The disease had lasted eight and a half months.

Pathologic Observations (five hours after death).—The gross findings were those of bronchopneumonia and hemorrhagic cystitis.

The brain appeared externally normal, but on the cut surface striking changes were observed in the region of the basal ganglions. Grossly, the globus pallidus, from its cephalic to its caudal limits, appeared to have been destroyed by a necrotic process (fig. 1). The lesion was bilateral and almost symmetric in distribution. The necrotic tissue was yellowish and soft and had partially sloughed off during fixation and successive manipulations. The margins of the lesion were ragged in appearance. The most necrotic region was the external segment, the internal segment being only partially involved. The lesion extended grossly beyond the external medullary lamina only in its most cephalic portion, where

the medial upper region of the putamen, adjacent to the globus pallidus, appeared involved (fig. 1). Likewise grossly necrotic was the lateral inferior portion of the head of the nucleus caudatus (fig. 1).

On microscopic examination, the necrotic areas consisted of a reticulum of fibers of varying size. In the Wilder and the Masson preparations these appeared to be connective in nature (fig. 2). Debris of nerve tissue was present, partly phagocytosed by gitter cells (compound granule cells). Myelin and axis-cylinders had disappeared. The blood vessels showed markedly thickened walls with fibrotic degeneration and partial hyalinization of adventitia and media (fig. 3). At the periphery of the necrotic area, the macroglia showed hypertrophic and hyperplastic changes as seen in sections prepared according to Cajal's method, but in the Holzer and Mallory preparations glia fibers were scanty and took little part in the formation of the meshwork which extended into the necrotic area.

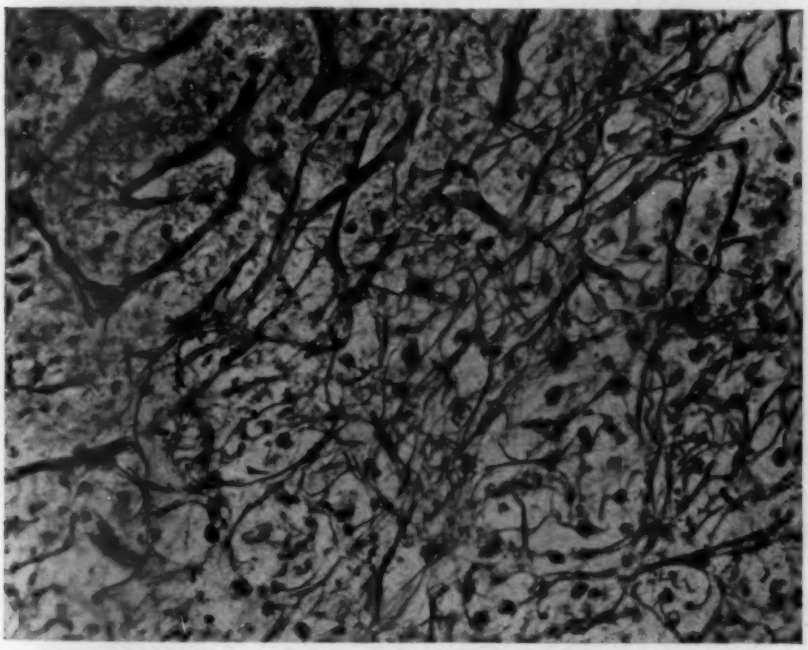

Fig. 2.—Connective reticulum within the necrotic area of the globus pallidus. Wilder's stain.

The involvement of the head of the nucleus caudatus and of the putamen was more extensive on microscopic examination than on gross inspection. Small foci of necrosis made up of gitter cells were scattered without order throughout the grossly normal portions of the basal nuclei, being usually perivascular in distribution. Outside the necrotic areas, the nerve cells were remarkably well preserved, but the neuroglia showed evidence of diffuse proliferation.

As to the cerebral cortex, at times no significant alterations were found in the available preparations with the exception of acute swelling of oligodendroglia and diffuse progressive changes of the macroglia, evidenced by a somewhat increased number of astrocytes, which were larger than normal. At other times, scattered about without order, microscopic areas of softening were seen. These, in their most advanced stages, appeared as circumscribed zones, roundish or

elongated in shape, in which both cellular elements and intercellular substance had been destroyed (fig. 4). A granular, fatty debris, partly included within compound granule cells, made up the necrotic zone, together with a coarse reticulum which stained well with Wilder's method and appeared to consist mostly of connective fibers. In the areas adjacent to these necrotic foci, the neuron cells exhibited varying degrees of cytoplasmic and nuclear changes, including the so-called ferruginous type, which was conspicuous in the larger cells. The microgliacytes were being transformed into compound granule cells, and the macroglia was hyperplastic and hypertrophic. Often a small blood vessel was found within the necrotic area. These foci were seen in all layers of the cortex but more frequently in the deep layers, where occasionally they extended lineally for a few millimeters, being then visible to the naked eye.

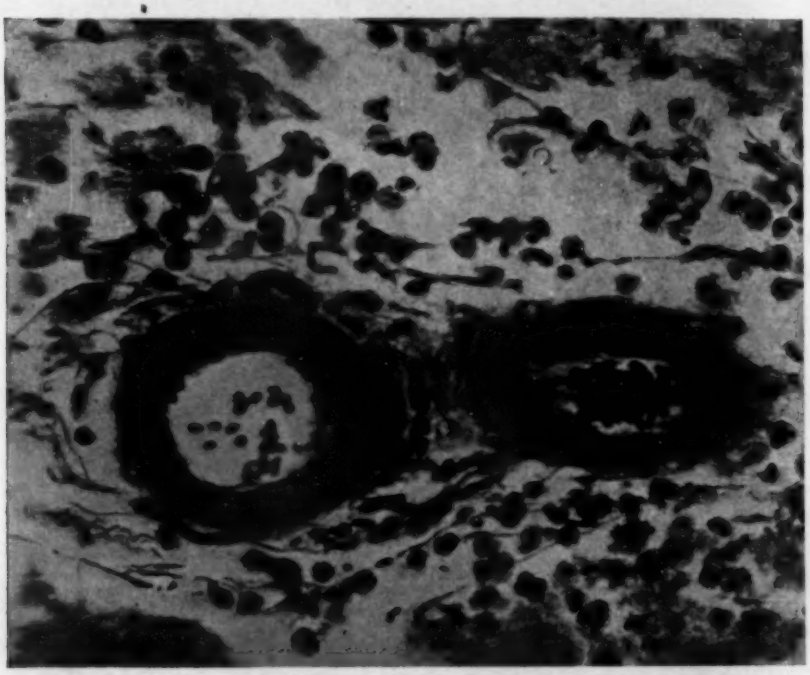

Fig. 3.—Blood vessels with thickened walls within the necrotic area of the globus pallidus. Masson's stain.

Less advanced degrees of this pathologic process consisted of circumscribed areas in which the cellular elements had disappeared but the tissue had not disintegrated. A few compound granule elements were found within the area, mainly about blood vessels.

The white matter of the hemispheres showed no gross changes. On microscopic examination, small necrotic foci were found occasionally scattered about without order or symmetry. They consisted of a microscopic area of demyelination (fig. 5), within which the myelin showed various stages of degeneration, gradually decreasing from the center toward the periphery. The area was filled with compound granule cells. A small blood vessel was usually present in the center of the demyelinated area.

Fig. 4.—Small necrotic area within the gray matter of the cerebral hemisphere. Masson's stain.

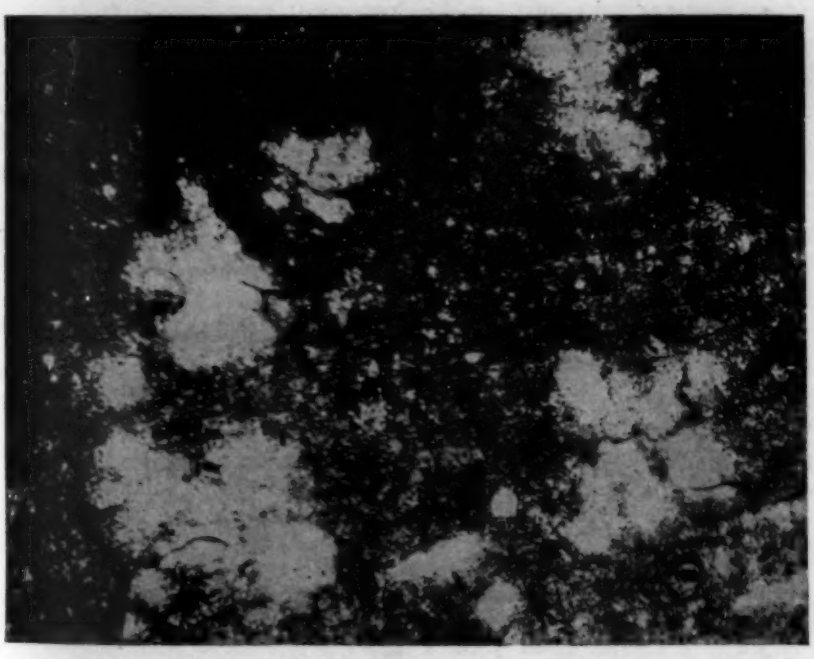

Fig. 5.—Small area of demyelination in the white matter of the cerebral hemisphere. Spielmeyer's stain.

In the cerebellum, on gross inspection, a few folia appeared somewhat skrunken and yellowish. Microscopically, these altered regions showed necrotic changes involving the molecular layer and the Purkinje cells. There were a large number of gitter cells in the necrotic area, and the overlying pia was thickened. The macroglia of the adjacent granular layer and of the underlying white matter showed marked proliferation with formation of numerous interlacing astrocytes and disappearance of myelin. Some cerebellar folia showed only incipient changes, characterized by partial necrosis of the molecular layer.

The thalamus and the brain stem showed no conspicuous pathologic changes. In the bulbar olives (inferior and superior olivary bodies) the neuron cells showed considerable degenerative lesions, being markedly reduced in number and exhibiting chromatolysis, swelling and shrinkage. In the Cajal preparation the neuroglia showed definite progressive changes.

In the spinal cord the pyramidal tracts appeared to have undergone degeneration, as evidenced by lack of myelin and presence of numerous globoid glia cells.

Histologic examination of liver, spleen, kidney and lung showed no significant changes.

COMMENT

There seems to be little doubt in the present case that the drugs administered were causally related to the development of encephalopathy. Which drug, barbiturate or opiate, played the major role in determining the pathologic changes is impossible to assess. However, it is reasonable to assume that the two drugs exerted an additive effect in damaging the brain, since their action is similar in character, as evidenced from a brief review of the pertinent literature.

Creutzfeld [1] observed severe degenerative changes of neurons, glia cells and blood vessels in a morphine addict who had taken large amounts of morphine and barbital sodium ("veronal") in a suicidal attempt and had survived sixty hours. Four cases of morphine intoxication, in 2 of which the condition was acute (survival periods two hours and forty-eight hours, respectively) and in 2 chronic, were described by Weimann.[2] The neuropathologic picture was one of diffuse neurocellular degeneration. In one of the 2 acute cases the pallidum contained ischemic areas of degeneration. A further case was reported by Weimann and Marenholtz.[3] The patient died six weeks after overdosage of morphine. Post mortem there was bilateral softening of the basal ganglions. Recently Swain [4] reported on the histologic examination of 3 patients in whom morphine intoxication had varied in duration. Acute and chronic neuronal degeneration were present together with irregular perivascular demyelination. In experimental morphine intoxication the classic findings of neuronal degeneration, first claimed by Nissl,[5] were

1. Creutzfeld, H. G.: Ztschr. f. d. ges. Neurol. u. Psychiat. **101**:97, 1926.
2. Weimann, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **105**:705, 1926.
3. Weimann, W., and Marenholtz, F.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **12**:297, 1928.
4. Swain, J. M.: Am. J. Psychiat. **102**:378, 1945.
5. Nissl, F.: Centralbl. f. Nervenh. **19**:544, 1896.

both confirmed [6] and denied.[7] Acute swelling of oligodendroglia was demonstrated by Wolff, Reed and Cobb.[8]

In regard to barbiturate intoxication, Petri [9] described small areas of demyelination and perivascular degeneration occurring in the brain of a patient who died three days after overdosage of barbital ("veronal"). Degeneration of neurons, particularly in the thalamus and the pallidum, together with vascular changes, were found by Scheidegger [10] in several cases of barbiturate poisoning. Occasional cerebellar lesions are also described, similar in nature to those found in the present case. In the patients dying of acute barbital intoxication described by Hassin,[11] aseptic meningitis, edema of the brain and widespread neuronal changes were the outstanding features. De Groat [12] put on record, without histologic examination, the finding of symmetric softening of the pallidum in a patient who died five days after overdosage of phenobarbital. In experimental barbiturate intoxication, degenerative changes of the neuron cells, either diffuse [13] or limited to certain areas,[14] and early demyelination [15] have been described.

It seems, in conclusion, that toxic doses of either morphine or barbiturates may bring about widespread degenerative changes of nerve cells, vascular lesions and occasionally focal necrosis. In the present case, focal necrosis was the outstanding pathologic feature, while neurocellular lesions were inconspicuous and limited mainly to the necrotic areas. It will be noted that in our case the period of survival after acute intoxication is the longest on record. It may be reasonable to assume that in some instances reported in the literature the period of survival was not long enough to allow necrosis to develop.

Some understanding of the mechanism by which these necrotic changes are brought about may be obtained by comparing the lesions observed in the present case with the similar type of pathologic changes

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6. Ma, W. C.: Chinese J. Physiol. **5**:251, 1931. Weimann.[2] Horning, E. S.: Am. J. Path. **10**:219, 1934. Weimann and Marenholtz.[3] Swain.[4] Nissl.[5]

7. McEwen, E. M., and Buchanan, A. R.: Arch. Neurol. & Psychiat. **36**: 1112, 1936.

8. Wolff, H. G.; Reed, W. P., and Cobb, S.: Arch. Neurol. & Psychiat. **21**: 1387, 1939.

9. Petri, E.: Virchows Arch. f. path. Anat. **284**:84, 1932.

10. Scheidegger, S.: Schweiz. Arch. f. Neurol. u. Psychiat. **39**:388, 1937.

11. Hassin, G. B.: Arch. Neurol. & Psychiat. **42**:679, 1939.

12. De Groat, A.: Arch. Path. **29**:271, 1940.

13. Mott, F. W.; Woodhouse, D. L., and Pickworth, F. A.: Brit. J. Exper. Path. **7**:325, 1926. Nakamura, T.: Tr. Soc. path. jap. **23**:487, 1933. Hartman, F. W.: Am. J. Clin. Path. **8**:629, 1938. Seevers, M. H., and Tatum, A. L.: J. Pharmacol. & Exper. Therap. **42**:217, 1931.

14. Hoff, H., and Kauders, O.: Ztschr. f. d. ges. Neurol. u. Psychiat. **103**: 176, 1926.

15. Hurst, E. W.: Australian J. Exper. Biol. & M. Sc. **20**:297, 1942.

found in other intoxications. Bilateral necrosis of the globus pallidus is a characteristic lesion of carbon monoxide poisoning, but it is occasionally found in intoxication due to other substances, including nitrous oxide,[16] potassium cyanide,[17] sodium azide [18] and carbon disulfide,[16] which are well known anoxic agents. Barbiturates, which are known to inhibit oxidation of glucose, lactate and pyruvate in brain slices in vitro [19] may be considered as conducive to anoxia. There is some evidence, therefore, indicating that anoxia is a common factor underlying necrosis of the globus pallidus.

Much discussion may be found in the medical literature as to whether the anoxic agent produces these necrotic phenomena by acting directly on the nervous parenchyma or whether the blood vessels are the primary site of injury, the parenchymatous changes being secondary to vascular damage. In the present case the perivascular location of many necrotic foci and the mesodermic reaction within the necrotic area seem to indicate a vascular origin of the lesion. Structural changes of the walls of blood vessels were prominent, however, only within the necrotic areas. Moreover, throughout the central nervous system, diffuse progressive changes of the neuroglia were observed, a finding which has long been associated with anoxia occurring, at times, without apparent damage of the vessel walls. There seems to be sufficient histologic evidence, therefore, to assume that the anoxic changes of the nervous parenchyma were brought about both by direct action of the toxic substance and through damage of the blood vessels.

The pallidum, whether because of peculiarities of vascular pattern or because of inherent metabolic individuality, appears to be selectively vulnerable to anoxia. However, when the intoxication and consequent anoxia are of considerable intensity, as in the present case, necrosis may occur also in other parts of the central nervous system.

SUMMARY

The case of a patient in whom, following overdosage of a barbiturate and an opiate, symptoms of extensive damage of the brain developed is described. Post mortem, bilateral necrosis of the basal ganglions was found. In addition, small necrotic foci were present throughout the cerebral hemispheres and the cerebellum. These changes are considered to result from anoxia which is brought about through damage of the blood vessels and by direct action of the narcotics on the nervous parenchyma.

16. Courville, C. B.: Medicine **15**:129, 1936. Sugge, W. D.: Virginia M. Monthly **70**:513, 1943.

17. Meyer, A.: Ztschr. f. d. ges. Neurol. u. Psychiat. **143**:333, 1933. Hurst, E. W.: Australian J. Exper. Biol. & M. Sc. **18**:201, 1940.

18. Richter, R.: J. Neuropath. & Exper. Neurol. **4**:324, 1945.

19. Jowett, M., and Quastel, J. H.: Biochem. J. **31**:565, 1937.

EFFECT OF CHOLINE IN THE PREVENTION OF EXPERIMENTAL AORTIC ATHEROSCLEROSIS

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IT IS NOW well established that choline is effective in preventing the deposition of fat in the livers of depancreatized dogs and rats fed diets high in fat or cholesterol. Choline is also effective in accelerating the removal of such lipid deposits after they have been formed. Hershey [1] first showed that lecithin has a striking effect on the removal of fat from the livers of depancreatized dogs maintained with insulin. Best, Ferguson and Hershey [2] demonstrated that this action of lecithin is due to its choline content. Since that time, numerous observers [3] have confirmed the lipotropic activity of choline, as well as of choline derivatives, on the fatty deposits in the livers of dogs and rats. More recently Herrmann [4] has shown that choline reduces the level of cholesterol and cholesterol esters in the serum, the aorta, the heart muscle and the liver in old hens.

The present study was designed to determine the effect of choline on the experimental atherosclerosis produced in rabbits by cholesterol feeding. Preliminary reports [5] from this laboratory suggested that choline exhibits a temporary action in preventing the development of experimental aortic atherosclerosis and that it may, in some instances, cause reabsorption of atheromatous lesions previously induced.

Baumann and Rusch [6] failed to observe this "protective action." However, in their experiment the relative proportion of choline to

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From the Research Service, First (Columbia University) Division, Goldwater Memorial Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.

1. Hershey, J. M.: Am. J. Physiol. **93**:675, 1930.
2. Best, C. H.; Ferguson, G. C., and Hershey, J. M.: J. Physiol. **79**:94, 1933.
3. Channon, H. J., and Wilkinson, A.: Biochem J. **28**:2026, 1934. Kaplan, A., and Chaikoff, I. L.: J. Biol. Chem. **120**:1647, 1937. Axyward, F. X., and Holt, L. E.: ibid. **121**:61, 1937.
4. Herrmann, G. R.: Proc. Soc. Exper. Biol. & Med. **61**:302, 1946.
5. Steiner, A.: Proc. Soc. Exper. Biol. & Med. **38**:231,1938; **39**:411, 1938.
6. Baumann, C. A., and Rusch, H. P.: Proc. Soc. Exper. Biol. & Med. **38**: 647, 1938.

cholesterol was less than that fed in the work just cited.[5] Moreover, their animals were maintained on the diet for one hundred and twenty days, a time long enough to mask the delaying action of the choline. Himsworth[7] reported that the characteristic atherosclerotic lesions appeared in the aorta of 1 animal killed on the forty-eighth day and 1 killed on the seventieth day of cholesterol and choline feeding. This result may have been due to the addition to the diet of sunflower seed oil, which has been shown to facilitate the absorption of cholesterol from the intestinal tract. However, Kesten and Silbowitz[8] showed that the feeding of soya lecithin or choline was effective in diminishing the incidence of experimental sclerosis in rabbits fed high cholesterol diets. Andrews and Broun[9] also reported that choline prevents the development of experimental cholesterol atherosclerosis in rabbits.

METHOD

Fifty-four male chinchilla rabbits approximately 6 months old were used. The animals were kept indoors in individual cages. The diet consisted of oats and fresh vegetables. One gram of cholesterol was mixed into the food of each rabbit three times weekly. The animals were divided into two groups. Group 1 consisted of 25 rabbits fed supplements of cholesterol alone. The remaining 29 animals, designated as group 2, were fed choline hydrochloride in addition to the cholesterol. Eighteen animals in group 2 were fed 500 mg. of choline hydrochloride daily, whereas 11 (nos. 609 through 649) were fed 1 Gm. daily. For administration, the choline hydrochloride was dissolved in approximately 5 cc. of water and then mixed with oats and ground carrots or cabbage. Food was withheld from the animals for four hours before they were fed cholesterol and choline hydrochloride, to insure ingestion. Blood was obtained from the ear veins at biweekly intervals. Cholesterol determinations were made on the whole blood by the method of Bloor, Pelkan and Allen.[10]

The rabbits died or were killed at periods from forty to one hundred days after the beginning of the experiment. An autopsy was done in each case, and the extent of fatty change in the aorta and the liver as shown macroscopically was noted and graded from 0 to 4 plus. One plus atherosclerosis was considered to be present when several elevated plaques approximately 3 mm. in diameter were observed in the aortic arch. Four plus atherosclerosis was designated when the entire aorta was covered with lesions. Two and three plus atherosclerosis were between these extremes.

All except 3 of the animals exhibited an immediate and progressive rise of the level of blood cholesterol; the animals that did not show a significant rise were nos. 150, 159 and 162 of group 1. It is known that an occasional rabbit, usually a male, is resistant to cholesterol feeding and does not show either hypercholesteremia or lipoid infiltration of the tissues.

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7. Himsworth, H. P.: Acta med. Scandinav., 1938, supp. 90, p. 158.

8. Kesten, A. D., and Silbowitz, R.: Proc. Soc. Exper. Biol. & Med. **49**: 71, 1942.

9. Andrews, K. R., and Broun, G. O.: Proc. Am. Soc. Clin. Investigation **19**:786, 1940.

10. Bloor, W. R.; Pelkan, K. E., and Allen, D. H.: J. Biol. Chem. **52**:191, 1922.

There was no appreciable difference in level of blood cholesterol between group 1 (cholesterol fed) and group 2 (choline and cholesterol fed). The blood cholesterol values for each rabbit are included in tables 1 and 2.

OBSERVATIONS

Gross Appearance of Tissues.—Group 1 and 2 animals were killed and examined in pairs at five to ten day intervals from the fortieth to the hundredth day of the experiment. The extent of gross lipoid infiltration of the aorta and the liver is recorded in the tables. In group 1 only 4 of the 25 animals were free of gross

TABLE 1.—*The Blood Cholesterol Values and Autopsy Observations in Group 1, Control Rabbits Fed Cholesterol Alone*

	Blood Cholesterol, Mg. per 100 Cc., at End of Given Number of Weeks							Feeding	Gross Fatty Changes	
Rabbit	0 Wk.	2 Wk.	4 Wk.	6 Wk.	8 Wk.	10 Wk.	12 Wk.	Days	Aorta	Liver
157	146	227	250	411	...	...	...	45	+	+
306	178	312	632	625	...	...	...	45	+	++
305	156	274	454	621	...	...	...	50	0	+
107	156	454	666	1,315	...	...	...	55	++	+++
156	145	250	192	495	555	...	...	57	+	+
262	162	333	492	602	704	...	...	60	++	++
272	126	271	406	588	1,234	...	...	65	++	++
160	113	367	400	421	746	...	...	68	+++	++
147	125	238	357	413	...	456	...	70	+	++
258	178	546	714	625	...	909	...	70	++++	++
264	136	450	725	1,250	...	1,388	1,224	80	+++	++
149	116	161	357	621	...	409	632	90	+	++
246	178	622	909	877	...	980	943	90	++	++
150	139	161	206	167	...	292	184	100	0	0
152	119	515	819	1,036	...	1,527	1,694	100	++	++
154	148	476	500	672	...	476	434	100	+	+
159	113	267	312	250	172	156	175	100	0	0
162	100	150	131	104	238	227	185	100	0	0
257	156	416	454	833	847	1,000	1,204	100	+++	++++
248	156	500	...	500	...	454	454	100	+++	++
345	210	625	...	632	...	625	884	100	+++	++
249	147	333	...	561	...	463	456	100	++++	++
405	125	454	...	501	...	629	729	100	+++	+++
488	178	633	...	450	...	581	567	100	++++	++++
509	123	225	...	228	...	333	301	100	+++	+++

Summary

Rabbits	Atherosclerosis
4	0
6	1+
5	2+
7	3+
3	4+

aortic atherosclerosis, while in group 2, 17 of the 27 animals failed to exhibit the characteristic aortic lesions. In group 1 the aortic atherosclerosis was graded as follows: 1 plus in 6 animals; 2 plus in 5 animals; 3 plus in 7 animals; 4 plus in 3 animals. The incidence of severe atherosclerosis was much lower in the choline-fed group: the lesions were graded as 1 plus in 7 animals, 2 plus in 4 animals and 3 plus in 1 animal. No animal in this group was found to have 4 plus lesions. The autopsies of animals killed from the fortieth to the eightieth day of the experiment revealed a more striking difference between the two groups (chart). None of the 14 rabbits fed choline hydrochloride and killed between the fortieth and the eightieth day showed macroscopic lipoid infiltration of the aorta. In group 1, 10 of 11 animals killed during the same period on corresponding days exhibited characteristic gross atherosclerosis. All of the rabbits receiving 0.5 Gm. of choline hydrochloride per day and examined after the ninetieth day showed lesions. How-

ever, of the rabbits receiving 1 Gm. of choline, the 2 examined on the ninetieth day and 2 of the 5 examined on the hundreth day were free of lesions.

The extent of lipoid infiltration of the liver, the adrenal gland and the kidney was similarly less in group 2, although the differences were not as great as in the case of the aorta.

TABLE 2.—*The Blood Cholesterol Values and Autopsy Observations in Group 2, Rabbits Fed Cholesterol and Choline Hydrochloride*

	Blood Cholesterol, Mg. per 100 Cc., at End of Given Number of Weeks								Gross Fatty Changes	
Rabbit	0 Wk.	2 Wk.	4 Wk.	6 Wk.	8 Wk.	10 Wk.	12 Wk.	Feeding Days	Aorta	Liver
Group 2a. Rabbits Fed Cholesterol and 500 Mg. Daily of Choline Hydrochloride										
120	125	454	970	Died				40	0	0
312	124	416	574	416	...	...	...	45	0	+
125	134	161	543	729	...	...	...	45	0	0
315	137	586	521	699	...	...	...	50	0	+
313	144	538	662	714	...	...	...	60	0	++
320	137	268	357	584	666	...	...	65	0	+
127	129	296	666	550	417	...	...	70	0	0
142	125	238	526	740	611	...	...	70	0	+
318	147	476	434	333	680	...	...	70	0	+
319	178	568	714	833	1,111	1,640	...	80	0	+++
145	91	277	746	1,000	1,667	1,000	...	90	+	+
316	135	381	833	673	629	588	...	90	+	++
126	100	274	641	888	833	769	1,129	100	+	+
129	117	208	385	909	909	793	1,117	100	+	+
124	102	108	112	208	278	671	512	100	+	+
140	125	384	555	719	833	1,219	2,739	100	+++	+++
146	119	227	781	707	505	718	784	100	+	+
317	148	293	315	351	382	724	...	100	++	++
314	169	208	485	454	561	699	...	100	++	++
Group 2b. Rabbits Fed Cholesterol and 1 Gm. Daily of Choline Hydrochloride										
612	119	695	307	532	...	...	...	50	0	++
609	132	250	340	...	...	...	...	60	0	0
616	142	108	252	409	526	421	...	80	0	+
617	156	489	708	659	678	462	421	90	0	++
640	106	125	276	344	390	390	483	90	0	+
613	110	333	426	600	695	916	...	100	0	++
648	125	390	695	623	1,063	1,785	...	100	0	+++
606	118	476	556	781	641	564	641	100	++	+++
611	208	806	714	1,067	746	247	806	100	+	+++
619	156	575	575	1,086	1,160	1,368	1,290	100	++	+++

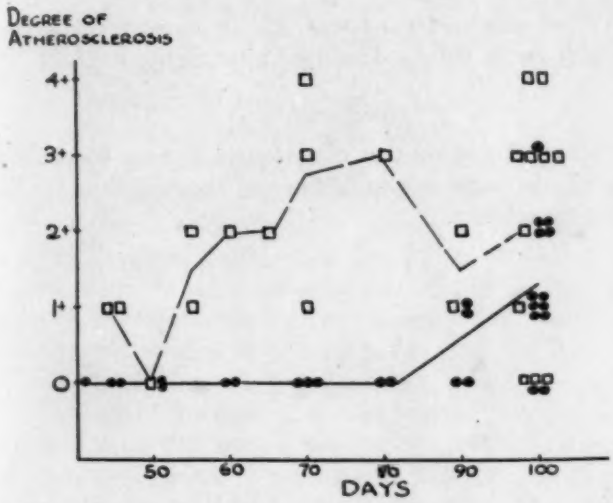
Summary

Rabbits	Atherosclerosis
17	0
7	1+
4	2+
1	3+
0	4+

COMMENT

The results of these experiments indicate that choline delays the development of atherosclerosis in rabbits fed large amounts of cholesterol. The finding that increasing the daily dose of choline hydrochloride from 500 mg. to 1 Gm. further delays the aortic atheroma suggests that a quantitative relation exists between the amount of choline hydrochloride fed and the time of the appearance of the lipoid infiltration of the aorta. It might be possible to prevent the atherosclerosis if the amount of choline hydrochloride was increased still more, or if the amount of cholesterol fed was decreased.

The protective action of choline in delaying the appearance of arterial lesions is not due to suppression of hypercholesteremia. In animals receiving choline the blood cholesterol levels were at least as high as in the control animals fed cholesterol alone. In this respect choline differs from thyroid extract and iodine,[11] which exert a protective action by preventing the development of hypercholesteremia in rabbits fed cholesterol. It is not known how choline acts in delaying the appearance of the arterial lesions. Some clue to the cause of its activity may be obtained when the mode of action of choline in preventing or curing fatty livers in dogs and rats has been definitely established. Welch [12]



Comparison of groups 1 and 2 with respect to the development of atherosclerosis. Squares represent rabbits of group 1 (fed cholesterol); circles, rabbits of group 2 (fed cholesterol and choline hydrochloride). The broken line represents the average degree of atherosclerosis observed in group 1; the solid line, the average degree in group 2.

and later Stetten [13] showed that dietary choline is used in direct synthesis of phospholipid by the liver itself. Perlman and Chaikoff,[14] using radioactive phosphorus as an indicator, reported that choline accelerates the synthesis and transfer of phospholipids in rats fed high fat diets and that the increase in phospholipid metabolism is proportional to the amount of choline ingested.

11. Turner, K. B.: J. Exper. Med. **58**:115, 1933.
12. Welch, A. D.: Proc. Soc. Exper. Biol. & Med. **35**:107, 1936.
13. Stetten, O. W., Jr.: J. Biol. Chem. **140**:143, 1941.
14. Perlman, I., and Chaikoff, I. L.: J. Biol. Chem. **127**:211, 1939

SUMMARY

In 29 rabbits fed 0.5 or 1 Gm. of choline hydrochloride daily in addition to 1 Gm. of cholesterol three times weekly, hypercholesteremia developed, comparable to that produced in 24 control animals fed cholesterol alone.

None of the choline-treated animals killed at intervals between the fortieth and eightieth days showed macroscopic atheroma in the aorta. Ten of 11 control rabbits fed the same amount of cholesterol and killed in similar periods showed gross atherosclerosis. Two animals receiving 1 Gm. of choline hydrochloride daily which were killed after ninety days, also 2 of 5 examined after one hundred days, were free of gross aortic lesions.

The extent of atherosclerosis was less in those animals receiving choline in which aortic lesions developed after eighty days.

CONCLUSION

Choline exhibits an action that is protective against the development of experimental atherosclerosis in rabbits fed cholesterol under the conditions of this experiment.

STUDIES ON THE MEGAKAROCYTE

I. The Normal Granulopoiesis of the Megakaryocyte

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WRIGHT'S [1] THEORY of the origin of the thrombocytes was founded (1) on the supposition that the granules seen in the cytoplasm of the mature megakaryocyte were identical in appearance and staining reactions with the chromomeres of the platelets and (2) on the assumption that ameboid pseudopodia protruding through the sinus walls disintegrated into platelets. Ogata,[2] Seeliger [3] and Frank [4] broadened this concept, asserting that strands and "tail-like" lumps may also separate from the megakaryocyte in situ and break up into platelets. This statement complies with Wright's original theory so far as the maturity of the megakaryocyte is presumed. The original concept, however, no longer prevails when free nuclei of megakaryocytes with platelets attached are also considered as evidence that thrombocytopoiesis is a function of the megakaryocyte. Most of these nuclei, according to size and features, belong to promegakaryocytes, which are as yet either free of granules or just showing beginning granulopoiesis. Willi [5] and Rohr and Koller [6] thought that they circumvented this discrepancy by the assumption that the granules of the platelets may also originate from chromatin expelled in the form of buds and small particles from the nuclei. These particles, on entering into protrusions of the cytoplasm, transform the latter into platelets. The authors thus returned to the theory of a nuclear origin of the granulation of the megakaryocyte, first expressed by Downey.[7] Other observations showing nuclei surrounded by masses of platelets without any trace of residual cytoplasm

From the Department of Hematologic Research, Medical Research Institute, Michael Reese Hospital. The Department of Hematologic Research is supported in part by the Hematology Research Foundation and the Michael Reese Research Foundation.

1. Wright, J. H.: Boston M. & S. J. **154**:643, 1906.
2. Ogata, J.: Beitr. z. path. Anat. u. z. allg. Path. **52**:192, 1912.
3. Seeliger, S.: Folia haemat. **29**:23, 1923; Klin. Wchnschr. **3**:731, 1924.
4. Frank, E.: Die haemorrhagischen Diathesen, in Schittenhelm, A.: Handbuch der Krankheiten des Blutes und der blutbildenden Organe, Berlin, Julius Springer, 1925, vol. 2, pp. 289-443.
5. Willi, H.: Folia haemat. **53**:426, 1935.
6. Rohr, K., and Koller, F.: Klin. Wchnschr. **15**:1549, 1936.
7. Downey, H.: Folia haemat. **15**:25, 1913.

were also interpreted as evidence of platelet formation by the megakaryocyte. All these assumptions disagree with the original theory in omitting the emphasis on maturity and on the significance of the pseudopodia and, furthermore, by presuming that the whole cytoplasm is suddenly and diffusely transformed into platelets. Moreover, they postulate a participation of the nucleus in the process of granulopoiesis. This variance of opinions induced a renewed study of the formation of the granules in the megakaryocyte.

OBSERVATIONS

Since in normal and many pathologic conditions the early developmental forms of the megakaryocytes are usually scarce, it was thought preferable to study bone marrows showing increased production of megakaryocytes. This may be found in chronic anemias with high normoblastic regeneration, in polycythemia vera and in those cases of thrombopenic purpura (Werlhof's disease) in which the megakaryocytes are numerous and show no inhibition of granulopoiesis. Numerous preparations of normal and pathologic marrows were used as controls.

In megakaryoblasts and early promegakaryocytes the first trace of granulopoiesis appears as a few granules of a more bluish or reddish color embedded in a spot of diminished density of the cytoplasm (fig. 1 *A*). This spot is always located close to the nucleus, opposite its indentation. An appearance of a different site results either from a perspective view or from mechanical distortion, and such appearances are therefore easily reduced to the typical pattern.

With the growth and development of the cell this area, which may be termed the "functional area," increases and assumes a pinkish color. The granules take the azurophilic stain more and more. Sometimes the area shows a rather sharp outline, but in most of the cells the outline, composed of less azurophilic or of basophilic granules, gradually changes into the basophilic cytoplasm. Nevertheless, the "functional area" can always be distinctly recognized by its color and diminished density (fig. 1 *B* to 1 *F*).

The "functional area" grows rapidly with the cell and involves more and more of the cytoplasm. Finally the latter becomes completely replaced by the granulated region except for a narrow hyaline border, stained transparent blue. With expansion the contour of the "functional area" becomes less distinct, so that the granulation tapers off toward the periphery (figs. 1 *H* and 2).

The growing "functional area" keeps the nucleus in an extremely eccentric position. Mechanical dislocation may obscure this fact. It is probable that the central location of the nucleus observed in some large adult megakaryocytes was artificially produced by the technic of smearing and also by liquefaction of the cytoplasm. In the well pre-

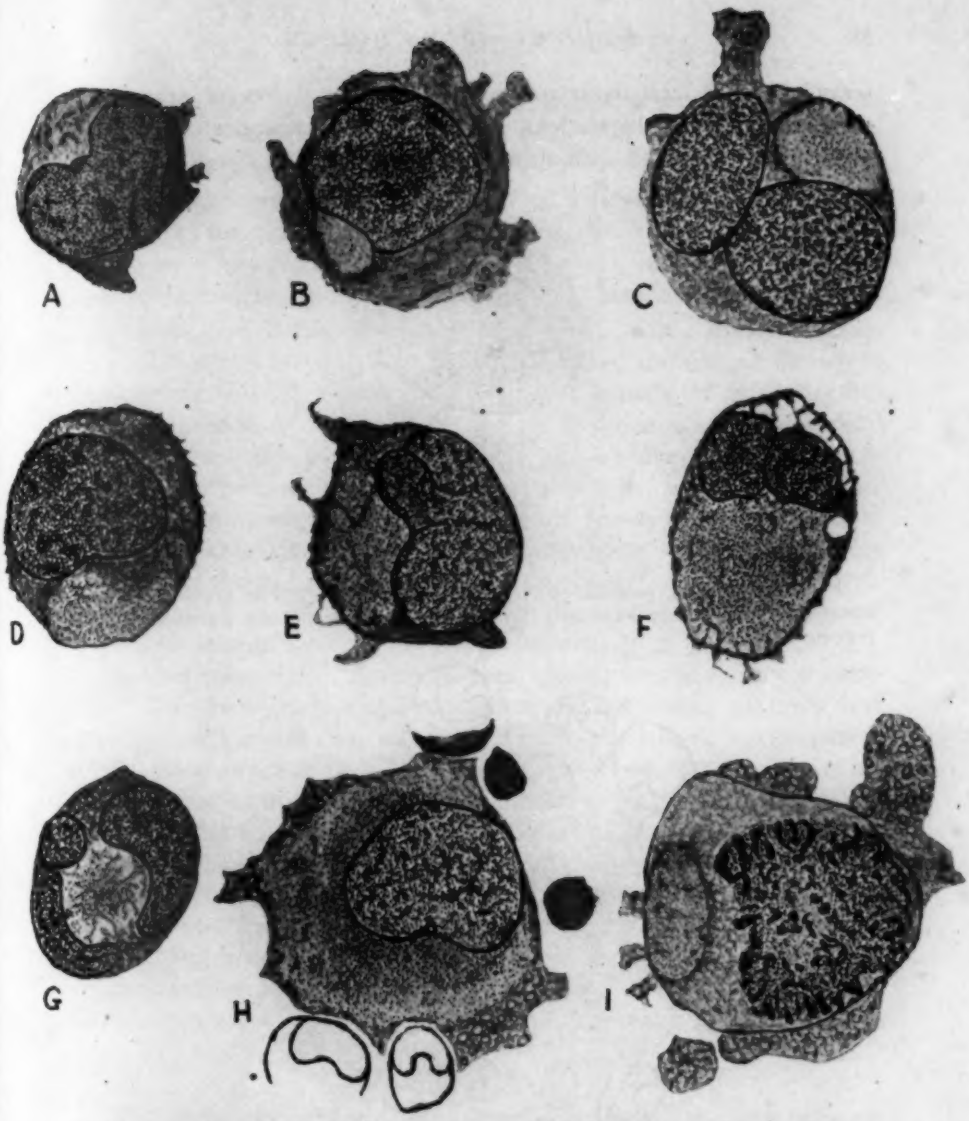

Fig. 1.—Normal granulopoiesis occurring in the development of the megakaryocyte. The reproductions are made from original drawings in colors.

A, very primitive megakaryoblast, 20 by 17.2 microns. It shows the "functional area" appearing at the indentation of the nucleus.

B, megakaryoblast, 24.5 by 24.5 microns. At this stage the "functional area" is well outlined and shows azurophilic granules.

C, binucleated promegakaryocyte, 30 by 27.5 microns. The "functional area" is growing.

D, promegakaryocyte, 27.2 by 21.5 microns. Note the lateral expansion of the "functional area."

E, promegakaryocyte, 27.5 by 21.5 microns. A more advanced phase is seen.

F, promegakaryocyte, 27.4 by 18 microns. The "functional area" occupies the cytoplasm on one side of the nucleus almost completely, leaving a narrow nongranulated zone, which surrounds the convexity of the eccentric nucleus.

G, promegakaryocyte, 21.5 by 17.2 microns. The "functional area" is in the center of a group of three nuclei.

H, megakaryocyte, 31.5 by 30 microns. Maturation is advancing. The granulopoiesis turns to the convex side of the eccentric nucleus.

I, promegakaryocyte in mitosis, 34.3 by 31.5 microns. The "functional area" preserves its outline, shape and size.

served cells the cytoplasm remains light blue and free of granules on the convex side of the nucleus, whereas in the remainder of the cell it may be entirely filled with them. Only very late in development may

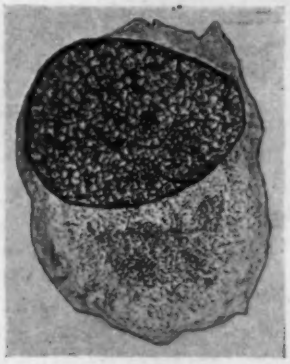

Fig. 2.—Maturing megakaryocyte, 45.8 by 40 microns. The granulation tapers toward the periphery, leaving a broad zone of ungranulated cytoplasm at the periphery.

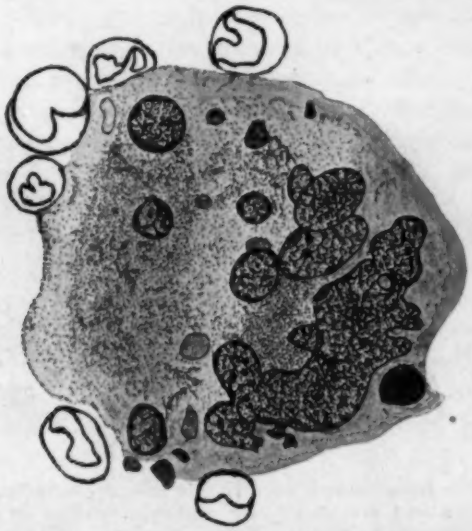

Fig. 3.—Adult megakaryocyte, 70 by 71.5 microns. The granulation fills the cell body almost completely and turns around the nucleus. A narrow rim at the convexity of the nucleus remains free of granules. The nucleus shows lobation and breaking off of larger and smaller lobes. Some of the latter, scattered through the cytoplasm, undergo pyknosis and become transformed into solid chromatin particles.

the aforementioned zone, also, become granulated. In some instances it can be distinctly recognized that the granulation is extending around the nucleus (fig. 3).

No instance was found which might suggest a formation of foci of granulopoiesis independent of the primary "functional area," or a diffuse onset of granulopoiesis, occurring throughout the cytoplasm.

In promegakaryocytes carrying three or four separated or coalescing nuclei the "functional area" is located in the center of the nuclear arrangement. Such a location is homologous with that already described, because the concavities of these nuclei are all turned inward (fig. 1 *G*).

The contour of the nucleus, whether this is either simple or complicated in shape, always remains sharply defined throughout the whole development. No budding or expelling of chromatin particles into the "functional area" was ever encountered in the absence of mechanical injury. When such particles do appear, they lie almost without exception on the convexity of the nucleus, especially where the nuclear membrane forms a part of the cell contour. Such partial denudations have to be considered artefacts. The separating of small and large lobules from the adult polymorphous nucleus belongs to the aging period. It takes place in the last phase of granulopoiesis and certainly has nothing to do with the origin of the granulation (fig. 3).

The behavior of the "functional area" during mitosis is very remarkable. The area remains quite uninfluenced in shape, structure and staining reaction and does not participate in the kinetic rearrangement of the cytoplasm (fig. 1 *I*). In these respects it reminds one of an inclusion body rather than of a living constituent of the dividing cell body. Yet the unbroken continuity of "functional area" and surrounding cytoplasm should prevent one from taking such an analogy too seriously. The separation of the "functional area" suggests the possibility that only one of the future daughter cells would carry the granulopoietic center. But this possibility is not realized since suppression of the segmentation of the cell body represents one of the essential steps in the development of the megakaryocyte and of giant cells in general.

COMMENT

The variations observed in the cells fit themselves quite naturally into successive phases of a continuous process. The beginning of the granulopoiesis in a distinct and definite region near the nucleus corresponds to the first appearing of secretory granules (Maximow [8]) at the site of the archoplasm and the Golgi apparatus in glandular cells. There is no visible sign of nuclear substances being expelled to take part either in the formation of the primitive "functional area" or in its growth. Heidenhain [9] saw the significance of the nucleus in the

8. Maximow, A.: Textbook of Histology, Philadelphia, W. B. Saunders Company, 1930, pp. 8 and 9.

9. Heidenhain, M.: Plasma und Zelle, in von Bardeleben, K.: Handbuch der Anatomie des Menschen, Jena, Gustav Fischer, 1907, vol. 1.

maintenance of the anatomic and functional characteristics of the cell. Therefore he objected to the view that the nucleus participates in the manufacture of functional structures and products of the cytoplasm by a disintegration of nuclear structures. More recent views acknowledge a functional activity of the nucleus, but a wide gap exists between such invisible cooperation and the expulsion of nuclear particles to be directly transformed into granules, which rise in fact from submicroscopic differentiations of the living substance.

The evidence suggested for the nuclear origin of the granulation of the megakaryocyte does not withstand criticism. Downey's [7] observations, rejected also by Naegeli,[10] concern mature megakaryocytes already showing abundant granulation. The mature megakaryocyte therefore appears unfit for valid deductions regarding the origin of the granules. The cells described by Downey show just a few extranuclear chromatin particles among the granules. Downey's investigations were performed on sections of fixed material and thus avoid the pitfalls of artefacts in smears. Yet one must consider that during the development of the megakaryocyte one or more pluripolar mitoses occur, in which irregular distributions of the chromosomes are frequent. Single or small groups of them become separated from the main mitotic figure and break up into small particles scattered throughout the cytoplasm. The aging nucleus of the megakaryocyte also undergoes degenerative changes like pyknosis, deformations and splitting off of lobes and lobuli, which may be transformed into homogenous chromatin lumps and particles. The assumption that the granules of the megakaryocyte originate from nuclear substance is therefore not established satisfactorily by Downey's investigations. Willi [8] and also Rohr and Koller [9] described promegakaryocytes without granulation which showed small cytoplasmic lobules at the periphery of the cell body and budlike nuclear excrescences. The latter were said to be severed off from the nucleus and transformed into granular substance, which, when entering into any of the lobules of the cytoplasm, gives rise to the chromomeres of the platelet, the hyalomere of which is formed by the lobule itself. The origin and the significance of these allegedly platelet-forming lobules will be discussed in another paper. But the beginning and development of the "functional area," as is to be understood from the descriptions given in the foregoing pages, provide reasons sound enough to reject any nuclear origin of the granules. Moreover, the staining reactions of the granules are not those of the chromatin. According to Roskin and Gruenbaum,[11]

10. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, ed. 5, Berlin, Julius Springer, 1931.

11. Roskin, G. O., and Gruenbaum, F. J.: Virchows Arch. f. path. Anat. **261**:528, 1926.

Rolla [12] and Undritz and Rothlin,[13] the chromomeres of the platelets do not give a positive Feulgen reaction of thymonucleic acid (desoxyribose nucleic protein). Gáspár [14] observed that nitric acid decalcification of fixed material destroys the chromatin but not the granules of the megakaryocytes. Whether the platelets' relatively high content of purine bodies claimed by Rohr and Koller [6] offers sufficient evidence of a nuclear origin of the chromomeres remains doubtful. This also applies to the figures simulating mitotic division, once advanced by Perroncito [15] for the nuclear nature of the chromomere and recently revived, though cautiously, by Estrada.[16]

Thus the granulopoiesis of the megakaryocytes is in full accord with the granular metamorphosis of other cells. The "functional area," formed entirely independently of any visible participation of nuclear structures at the typical site, retains this eccentric position until the stage of complete maturity of the cell is reached. The "functional area" involves the surrounding cytoplasm by concentric growth, never by coalescing with separately created foci of granulopoiesis. The process of transformation is continuous and monocentric. The continuous expansion of the "functional area" also explains the terminal granule-free rim and the eccentric position of the nucleus.

I should like to have confirmed my observations on sections of fixed marrow but so far have failed to obtain satisfactory results. The staining of the granules either with the Giemsa or other methods is precarious. Experience with the special method of Kingsley was also not encouraging. Another difficulty arises from the shrinking and the preservation of the three-dimensional extension of the cells, compared with the flattened cells of the smears. The size of the cells in the earliest stage of development is ordinarily so much reduced that the initial "functional area" becomes invisible. Yet rare instances of a somewhat older promegakaryocyte presenting a well recognizable "functional area" of typical location and form were found. Nevertheless, in sections as well as in smears a mature megakaryocyte having an apparently central nucleus is frequently encountered. Such pictures may result from the position of the longer axis relative to the plane of section or from a shifting of the nucleus to the center in a more liquefied cytoplasm either natural or brought about artificially by shrinking or the process of smearing.

The fact that the "functional area" is excluded from the cytoplasmic rearrangement during mitosis implies that with the growth of the granulated district the kinetic capacity of the cell decreases. This

12. Rolla, A.: Haematologica **16**:619, 1935.
13. Undritz, E., and Rothlin, E.: Helvet. acta med. **13**:595, 1946.
14. Gáspár, St.: Frankfurt. Ztschr. f. Path. **34**:460, 1926.
15. Perroncito, A.: Haematologica **2**:510, 1921.
16. Estrada, A.: Sang **10**:1088, 1936.

suggestion is supported by the experience that mitosis of completely granulated megakaryocytes was never observed. All mitotic figures belonged either to the megakaryoblasts or the promegakaryocytes or to somewhat more mature cells that had a significant residue of granule-free basophilic cytoplasm. Nevertheless, in the case of the megakaryocyte such inferences should be asserted with caution, since the suppression of the segmentation of the cell body in mitosis characterizes the development of the megakaryocyte and also that of other giant cells (Schwarz[17]). Whether, in spite of the exclusion of the mature cytoplasm from cytokinesis the nucleus may enter karyokinesis independently is a question that would demand special studies. It may be noted, however, that Kaufmann[18] in his careful studies of the growth and polymorphism of the nucleus of the mature megakaryocyte has never found reason to suppose mitotic or amitotic division in considering such nuclei.

The megakaryocyte was the object of numerous investigations after Wright[1] had proposed his theory. Yet the developmental history of the granulation received no special attention. Interest was almost exclusively centered on the manner in which the granules were related to platelet formation. Granulopoiesis in normal and in pathologic conditions was treated as a whole in respect of the preparatory arrangement of the granules and the transition through which these became the chromomeres of the platelets. Although this interest was also predominant in the recent studies of de la Fuente,[19] he discussed the development of the granulopoiesis. He stated expressly that the first granules appear in a distinct area of the cytoplasm of the megakaryoblast. In this area the cytoplasm shows diminished density and loses the intensive basophilia so characteristic of the early stages. This area usually lies in the vicinity of the nucleus. So far these observations agree well with mine, but differ in a most essential point, since de la Fuente asserted that multiple areas may appear in any part of the cytoplasm. The reason for this disagreement may be found in the fact that he rather vaguely determined the site of the area as near the nucleus without accentuating the biologic significance of this site in relation to the archoplasm. De la Fuente's descriptions also omit the intermediate stages between the first appearance of the area and the rather advanced granulopoiesis in promegakaryocytes. In such stages mechanical distortions and transpositions of the granulated material may possibly have produced deceptive pictures.

Fieschi and Villalobos[20] described granulopoiesis of the megakaryocyte as a diffuse process. With the appearance of the granules, the stain of the cytoplasm changes from basophilia to slight neutrophilia.

17. Schwarz, E.: Am. J. Anat. **79**:75, 1946.
18. Kaufmann, D. M.: Anat. Rec. **42**:365, 1929.
19. de la Fuente, V.: Arch. Int. Med. **79**:387, 1946.
20. Fieschi, A., and Villalobos, S. F.: Haematologica **20**:535, 1939.

This change is allegedly so rapid that transitional phases cannot be seen. Consequently they interpreted a promegakaryocyte (their fig. 1 *B*) with a granulated district as showing precocious granulopoiesis in a basophilic cytoplasm. Obviously the picture is that of a promegakaryocyte with a "functional area," which is already somewhat larger than that shown in figure 1 *C* of the present study. The cell presents a normal developmental stage, not abnormal, premature granulopoiesis. The transitional phases, missed by Fieschi and Villalobos, are the successive phases of the growing "functional area."

Summarizing the properties and the development of granulopoiesis, one finds that the megakaryocyte behaves just like any other cell characterized by specific functional transformation of its cytoplasm. The megakaryocyte was always considered a specific cell even before it was viewed in connection with platelet formation. Yet it does not seem superfluous to emphasize this specificity on the basis of the observations reported in this paper, because of the concept of Levy.[21] This author has assumed that the interruption of cytokinesis, which is the fundamental mechanism in the production of cellular gigantism, accounts for all the features of giant cells and deprives them of their specificity. Levy considered the suppression of segmentation of the cell body as a pathologic process—utilized in the bone marrow for a physiologic purpose, namely, the formation of the megakaryocyte. Should one not, better, reverse this proposition and say that a process physiologic in one type of cell is to be called pathologic when occurring in other cells? Moreover, I [17] have shown that gigantism due to suppressed cytokinesis does not influence the specific functional transformations of the cytoplasm in erythroblasts and myeloblasts.

Findings in pathologic conditions, reported in the following paper, agree in every detail with the concept of the significance of the "functional area" described in this paper. The observation that the normal and pathologic processes mutually correspond in all essential points enhances the validity of the presented interpretation.

SUMMARY

Granulopoiesis begins in the megakaryoblast in a well defined, distinct area, for which the term "functional area" is proposed. The "functional area" is located near the indentation of the nucleus, at the site of the archoplasm. By concentric and continuous expansion it gradually involves the entire cytoplasm with the exception of a narrow border zone, which remains free of granulation. The granulopoiesis is monocentric. Granulopoietic foci independent of the primary area apparently never develop. The position and the growth of the "functional area" keep the nucleus in an eccentric position. Nuclear structures do not visibly participate in the formation of the granules.

21. Levy, F.: Am. J. Clin. Path. **15**:154, 1945.

STUDIES ON THE MEGAKARYOCYTE

II. Deficient Granulopoiesis in the Megakaryocyte in Essential Thrombopenic Purpura

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CASES OF THROMBOPENIC PURPURA are usually divided into two groups: those of essential thrombopenic purpura (Werlhof's disease) and those of symptomatic or secondary thrombopenia. Ignorance of the cause and the pathogenesis in the first group represents the only mark of distinction. A classification based on a purely negative characteristic is bound to break down with every advancing step of knowledge and suggests that one deals, in Werlhof's disease, with a syndrome and not with a pathogenetic entity. The encouraging results of splenectomy closed the gap of knowledge but temporarily. In many cases the platelet count returned to the preoperative level in a rather short time; in a few others the operation had no influence at all. Therefore, the splenic factor allegedly inhibiting thrombocytopoiesis can no longer be regarded as the unifying connecting link. Similar discrepancies resulted from the study of the megakaryocytes, the assumed mother cells of the platelets.

In the great majority of the cases the megakaryocytes were increased in number without showing significant histologic changes. The presence of a higher proportion of early developmental forms of the megakaryocytes, frequently interpreted as an abnormal feature, is but the consequence of the increased rate at which the cells are produced from the reticulum. Granulopoiesis was not found wanting either in intensity or in the final arrangement of the granules preparatory to the segregation of platelets, the "pearl strands" of Ogata[1] and the checkered pattern (*Felderung*) of Seeliger.[2] I myself have observed the most exquisite examples of the latter distribution in many cases of essential thrombopenic purpura. Therefore, only the last steps of the formation of platelets were left as the point of attack. The protrusion of pseudopodia was found to be normal by Vogel, Erf and Rosenthal,[3] very marked

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1. Ogata, J.: Beitr. z. path. Anat. u. z. allg. Path. **52**:192, 1912.
2. Seeliger, S.: Folia haemat. **29**:23, 1923.
3. Vogel, R.; Erf, L. A., and Rosenthal, N.: Am. J. Clin. Path **7**:436 and 498, 1937.

by Görög,[4] scarce or absent by Jedlička and Altschuller,[5] Salzmann,[6] Dvorak[7] and Willi.[8] Much more in agreement are the observations concerning the possibility that platelets are segregated directly from the mature megakaryocyte. Appearances pointing to that activity, rare or absent before splenectomy, were reported to be numerous after the operation. It should be emphasized that when platelets are superposed on and apposed to immature and mature megakaryocytes fallacious appearances are produced, which obviously will be rare or absent in the thrombopenic period and will reappear with the increase of the number of platelets, especially at the time of the platelet crisis following splenectomy. This point will be detailed in another paper. Moreover, observations like that of Krjukof,[9] showing the bone marrow rich in platelets and the circulating blood containing few, are entirely incompatible with the concept of inhibition of platelet production.

In contrast with that most common type of essential thrombopenic purpura, in which there is an increased number of megakaryocytes, Minot,[10] Askanazy,[11] and Lawrence and Knutti[12] reported cases with reduced numbers, and Koehler,[13] Bykowa,[14] Ferrata and Storti[15] and Zitzmann[16] even observed complete absence of these elements.

Cases of Werlhof's disease differing from the common type by distinct lesions of the megakaryocytes merit special attention. Such observations were reported by Seeliger,[3] Frank,[17] Gáspár,[18] Jedlička and Altschuller,[5] Arinkin,[19] Stern and Hartmann,[20] Weiner and Kaznelson,[21] Schmincke,[22] Gerlach,[23] Görög,[4] Lawrence and Knutti,[12] Willi,[8]

4. Görög, D.: Virchows Arch. f. path. Anat. **284**:917, 1932.
5. Jedlička, V., and Altschuller, G.: Acta med. Scandinav. **64**:123, 1926.
6. Salzmann, S. R.: Ohio State M. J. **25**:792, 1929.
7. Dvorak, J.: Klin. Wchnschr. **10**:1077, 1931.
8. Willi, H.: Folia haemat. **53**:426, 1935.
9. Krjukof, A.: Sang **9**:363, 1935.
10. Minot, G. R.: Arch. Int. Med. **19**:1062, 1917.
11. Askanazy, M.: Schweiz. med. Wchnschr. **62**:681, 1932.
12. Lawrence, J. S., and Knuttl, R. E.: Am. J. M. Sc. **188**:37, 1934.
13. Koehler, G. D.: Klin. Wchnschr. **6**:314, 1927.
14. Bykowa, O.: Virchows Arch. f. path. Anat. **268**:606, 1928.
15. Ferrata, A., and Storti, E.: Gazz. d. osp. **59**:855, 1938.
16. Zitzmann, K.: Folia haemat. **56**:129, 1937.
17. Frank, E.: Die haemorrhagischen Diathesen, in Schittenhelm, A.: Handbuch der Krankheiten des Blutes und der blutbildenden Organe, Berlin, Julius Springer, 1925, vol. 2, pp. 289-473.
18. Gáspár, St.: Frankfurt. Ztschr. f. Path. **34**:460, 1926.
19. Arinkin, M. J.: Folia haemat. **38**:233, 1929.
20. Stern, R., and Hartmann, E.: Ztschr. f. klin. Med. **104**:490, 1926.
21. Weiner, W., and Kaznelson, P.: Folia haemat. **32**:233, 1925.
22. Schmincke: Verhandl. d. deutsch. path. Gesellsch. **25**:50, 1930.
23. Gerlach, G.: Centralbl. f. allg. Path. u. path. Anat. **48**:81, 1930.

Rohr,[24] Jaffé,[25] Fieschi and Villalobos,[26] Revol,[27] Limarzi and Schleicher,[28] Dameshek and Miller [29] and Valentine.[30] Cases of secondary thrombopenic purpura, in which the deficiency develops in the course of infections and intoxications, are not included in that enumeration. The changes of megakaryocytes in these observations are far from being uniform. They involve deficient granulopoiesis, vacuolation of the cytoplasm and denudation and degeneration of the nuclei of various types. Differences in the material used (obtained at necropsies or at biopsies) and in technics (dried or fixed smears, sections made after various fixations and prepared according to various staining methods, with either Romanowsky stains or current histologic dyes) make any comparison problematic. The only feature appearing in several cases and studied with reliable technic is a more or less pronounced deficiency of the granulation down to its complete absence. Even in this regard some of the descriptions are lacking in important details, and in the more accurate observations there is no indication of the nature of the condition, whether a mere suppression of the granulopoietic function or a degenerative process. Compared with the ever increasing number of observations of normal granulopoiesis, the instances in which visible cytologic changes were reported seem to be very rare. Among 26 cases of Werlhof's disease examined by me only 1 belonged to the latter group. This scarcity, the special type and extraordinary degree of the lesions and their manifest relation to the developmental phases of normal granulopoiesis described in the preceding paper may justify a detailed report of the observations.

OBSERVATIONS

E. B., a white woman 25 years old, had suffered from symptoms of a hemorrhagic diathesis since her sixteenth year. Hematologic findings from that period were not available. The diagnosis of Werlhof's disease had been made repeatedly. She was delivered of a normal infant on Sept. 20, 1946. The blood findings at term were: red cells, 2,750,000; white cells, 14,200; platelets, 30,000; bleeding time, thirty-five minutes. The spleen and the liver were not enlarged. A sternal puncture performed ten days later showed an active marrow, especially in respect to leukopoiesis, and very numerous megakaryocytes.

The striking features were a hyaline appearance of the great majority of the adult megakaryocytes and an increased proportion of promegakaryocytes and megakaryoblasts. The differential count of 400 cells showed 1.75 per cent mature granulated megakaryocytes, 62.25 per cent hyaline adult megakaryocytes and 33

24. Rohr, K.: Helvet. acta med. **1**:713, 1935.
25. Jaffé, R.: J. A. M. A. **107**:124, 1935.
26. Fieschi, A., and Villalobos, S. F.: Haematologica **20**:535, 1939.
27. Revol, L.: Sang **13**:884, 1939.
28. Limarzi, L. R., and Schleicher, E. M.: J. A. M. A. **114**:12, 1940.
29. Dameshek, W., and Miller, E. B.: Blood **1**:27, 1946.
30. Valentine, E. R.: Am. J. M. Sc. **214**:260, 1947.

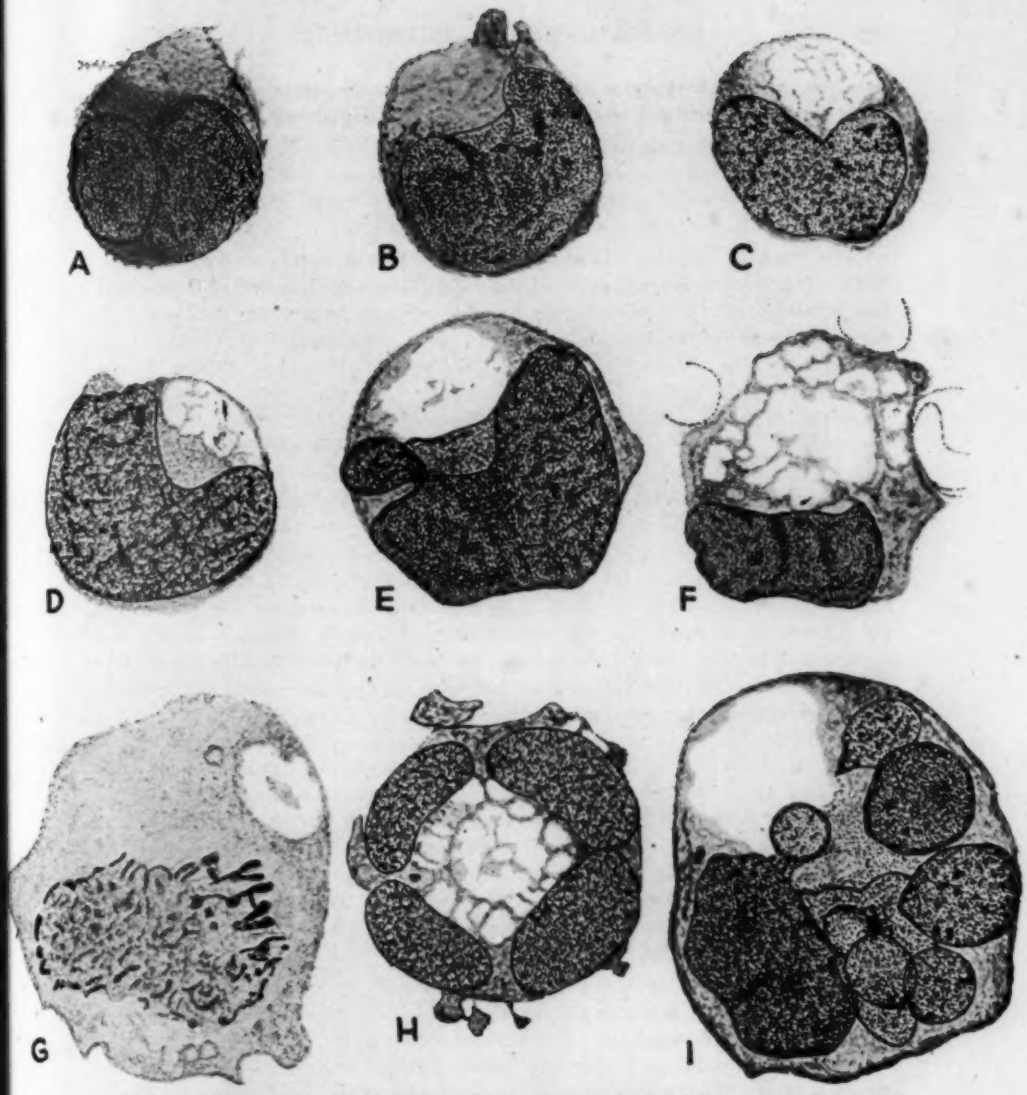

Fig. 1.—Granulopoiesis of the megakaryocyte as observed in a case of essential thrombopenic purpura. The reproductions are copied from original drawings in colors. *A*, early megakaryoblast, 24.5 by 23.5 microns, showing the first appearance of the "functional area."

B, megakaryoblast, 25.7 by 25.3 microns. There is beginning hyalinization of the "functional area."

C, promegakaryocyte, 25.7 by 21.5 microns. The growing "functional area" is more distinctly hyalinized. Note the basophilic filaments.

D, promegakaryocyte, 27.9 by 25.7 microns. The "functional area" assumes a vacuolar appearance.

E, megakaryocyte, 30 by 28.6 microns. It shows a large hyalinized "functional area" of vacuolar appearance.

F, promegakaryocyte, 21.9 by 31.4 microns. In the large hyaline "functional area" the meshwork of basophilic filaments forms a peripheral zone, similar to that observed in vacuolation.

G, promegakaryocyte in mitosis, 37.2 by 37.2 microns. The hyaline "functional area" preserves its outline, shape and size.

H, promegakaryocyte, 30 by 28.6 microns. The hyaline "functional area" rests in the center of a group of four nuclei.

I, polykaryocyte with large functional area, 42 by 42 microns.

per cent promegakaryocytes and megakaryoblasts. Separate counts of the two early types were omitted because of the arbitrary distinction.

The hyaline adult cells were large, frequently surpassing 70 microns. They were vulnerable, many of them being more or less mutilated, and showed no trace of granulation. To avoid repetition, detailed descriptions of the hyaline condition will be given in connection with the developmental stages.

The youngest megakaryoblasts showed for the most part a normal appearance. Only a few were striking because of their size. The nuclei were round or more or less indented; rarely the indentation was deep enough to separate the nucleus into two still connected or independent halves. The cytoplasm of the youngest cells stained deep blue. In very primitive megakaryoblasts there had already appeared on the side of the nuclear indentation a distinct area where the cytoplasm was of diminished density and contained well separated basophilic granules on a more homogeneous brighter blue background (fig. 1 *A*). This area was well circumscribed, and the zone of transition leading to the deep blue and denser cytoplasm was very narrow. With the growth of the cell, the area increased, and its basophilic granules became scarcer, more distant from one another and paler than before. Often they were arranged in beady filaments, which usually extended from the rim of the area into the central region (fig. 1 *B*). Later the light blue background changed into a colorless, hyaline ground substance (fig. 1 *C*). With the growth of the area, the filiform arrangement of the granules became more accentuated, but the size and the stain of the beads decreased rapidly (fig. 1 *D* and *E*). The filaments formed a network of wide meshes and recalled thereby the picture of vacuolar degeneration of a promegakaryocyte (fig. 1 *F*). But the topography distinguished the two types; in vacuolar degeneration the cavities always begin peripherally and spread around the circumference; the hyaline area described here always occupied the original site and grew by continuous concentric expansion (fig. 1 *D, E, F* and *I*; fig. 2 *C*). The shape of the area, the number of nuclei and the position in the optic axis sometimes influenced the apparent site of the area. In plurinuclear cells (fig. 1 *H*) the truly central position of the area did not differ principally from the primary site in the uninucleated cell. Phagocytic activity of promegakaryocytes of that type is shown in fig. 2 *A*, where a neutrophilic and an eosinophilic leukocyte may be seen included in vacuoles.

The ground substance of the hyaline area presented some variations in appearance and structure. Usually in the initial phase it showed a faint blue tinge, which rapidly faded in the progressive development and finally vanished. Simultaneously the basophilic granules and filaments became scarcer. Finally the area impressed one as a cavity filled with a homogeneous colorless fluid. But cells such as that shown in figure 2 *B* contradicted this assumption. In such cells the area was mechanically dislocated, almost excluded from the main cell body; yet it preserved outline and shape. This presumes a more solid physical condition, like that of a viscous mucus or a jelly. In other cells, especially those more intensively stained, a cloudy mass appeared in the center, composed of minute, dustlike particles, a product of precipitation or of coagulation due to the process of drying and fixation. This mass could not be interpreted as a precursor of the azurophilic granules. Yet, in later stages, indications of frustrated granulopoiesis sometimes appeared. In such instances the hyaline zone showed sometimes a slight pinkish hue and contained some granules of very faint staining, so that they were noticed only by the greatest attention and any reproduction was impossible. These features suggest that the cell had started functioning in the direction of granulopoiesis but achieved only a minimal amount of an abortive product.

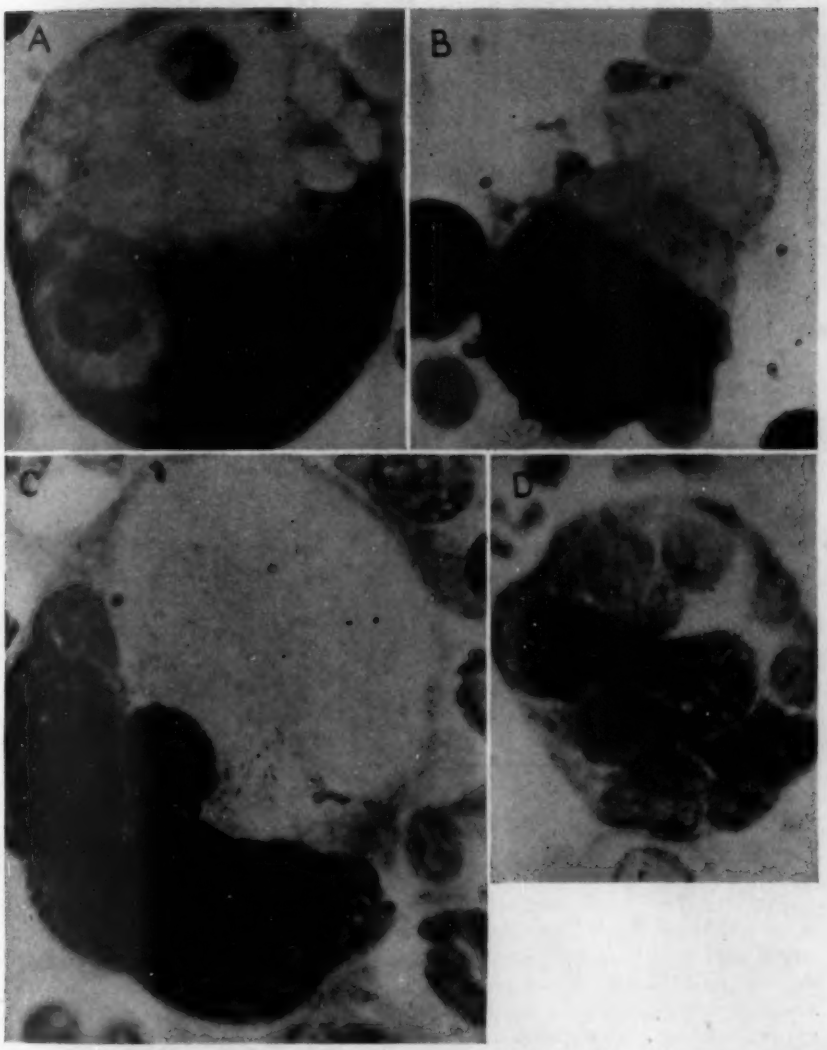

Fig. 2.—*A*, young megakaryocyte, 42.9 by 48.6 microns. Note hyalinization of the "functional area," inclusion of a neutrophilic and an eosinophilic leukocyte and vacuolar degeneration.

B, promegakaryocyte. The main cell body measures 25.7 by 25.7 microns. There is artificial separation of the "functional area."

C, adult megakaryocyte, 50 by 40 microns. The hyaline "functional area" occupies almost the entire cell body, leaving a thin border zone of basophilic cytoplasm.

D, polykaryocyte, 40 by 40 microns. The cytoplasm is hyaline; only a narrow basophilic border zone is left.

The hyaline area retained its form, outline and position during mitosis. It apparently did not partake in the kinetic rearrangement of the cytoplasm (fig. 1 *G*). The great number of megakaryocytes in the smears permitted one to follow the widening of the hyaline area in a most exact manner. Finally only a narrow blue zone was left, and the cell appeared almost totally hyalinized (fig. 2 *C*). Later the megakaryocytes became very large and their big nuclei very polymorphous. The aging cells transformed into polykaryocytes (fig. 2 *D*). Large, though smashed and deformed, mitotic figures (fig. 3 *A*) encountered on the smears suggest that the multiplication of the nuclei forming the polykaryocyte may have resulted not only from breaking of a single lobated nucleus but from karyokinesis without the regular fusion of the products of division. The finding of two such figures on one slide betrays a rather great mitotic activity when one considers the few mitoses encountered at earlier stages of the development.

These large mitotic figures appeared separated from any cell body. Only an extremely faint halo, which could not be reproduced in the picture, revealed the presence of a substance which kept the chromosomes together. Such free mitotic figures are, in my experience, also frequently seen in marrow with highly stimulated normoblastic or megaloblastic erythropoiesis and belong there to dividing reticulum cells, which proliferate intensively in such conditions. In the peripheral zone the cytoplasm is very delicate and extremely vulnerable, whereas in the field containing the chromosomes the ground substance is more resistant, since it contains the bulk of the achromatic substance. The smearing tears off the more fluid exterior zone, with the result that a comparatively undamaged free mitotic figure subsists. The hyaline cytoplasm of the large megakaryocyte seems to present analogous physical conditions.

The progressive developmental cycle ended with the polykaryocyte. The vitality of the cell decreased as shown by the fact that leukocytes migrated into the cell body (fig. 3 *B*). The lack of definite vacuoles and the preservation of the leukocytes, intact, oppose an interpretation of the picture as resulting from active phagocytosis on the part of the megakaryocyte. The dense accumulation of polymorphonuclear leukocytes seen around a very large megakaryocyte in figure 3 *C* and the great number of leukocytes within the disintegrating cell body provide satisfactory evidence of positive chemotaxis and invasion.

Another type of abnormal cells was represented by large megakaryocytes with a more or less basophilic cytoplasm containing no azurophilic granulation and a large single polymorphous nucleus or multiple nuclei, properly belonging to the mature stage. These cells showed no trace of a hyaline area. Such cells are also found in small numbers in the common form of Werlhof's disease and in many other conditions. This abnormality consists in a dissociation of cytoplasmic and nuclear maturation. Yet the polykaryocyte reproduced in figure 3 *D* exceeds such cells by the great number of nuclei and the very dark blue of a peculiar shade of the cytoplasm. Such cells have never been described hitherto, nor have they been seen in any other marrow by me.

COMMENT

The pictures illustrating step by step the process of hyalinization correspond to those of normal granulopoiesis, described in the foregoing paper, as the negative to the positive of a photographic film. The site of the hyaline region, its continuous concentric expansion and its behavior during mitosis repeat the successive developmental stages

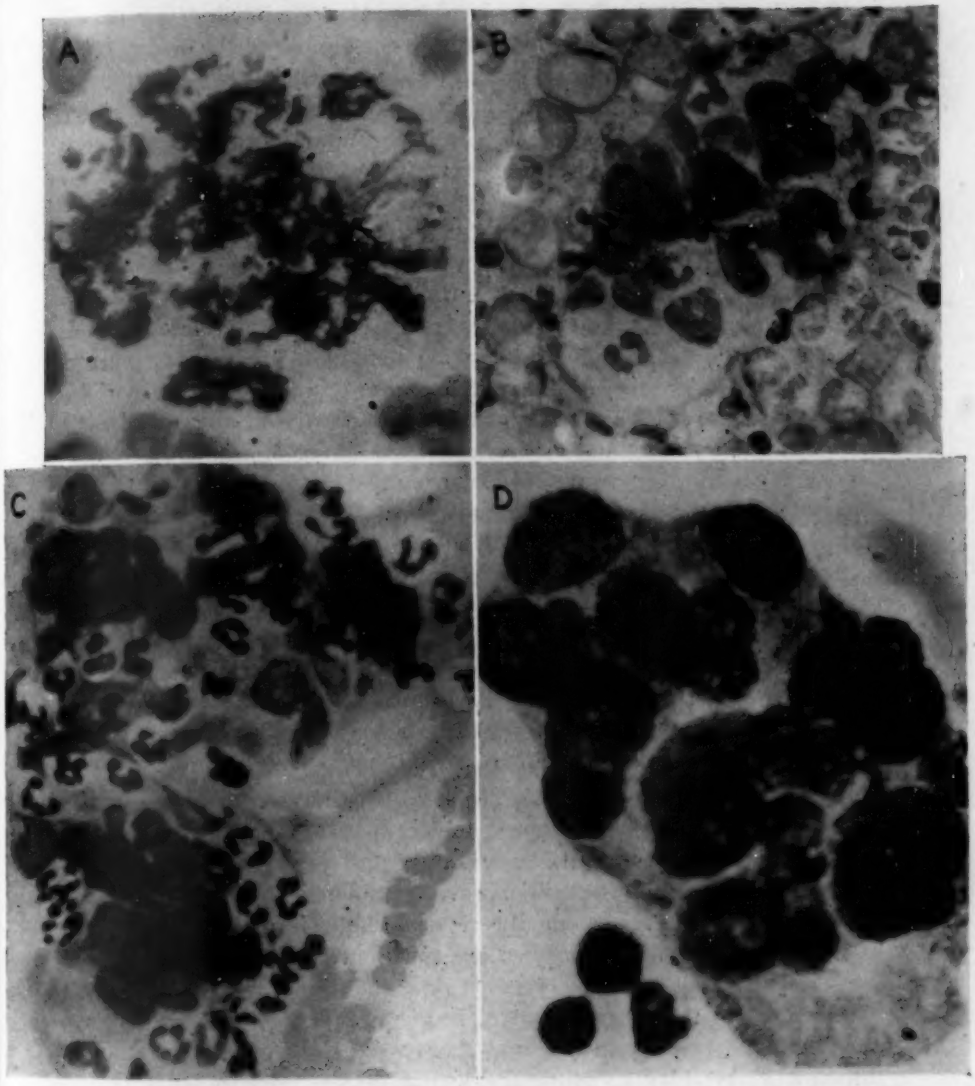

Fig. 3.—*A*, large pluripolar free mitosis, embedded in an achromatic, almost invisible substance.

B, huge, aged, hyalinized polykaryocyte, 102.9 by 58.6 microns, invaded by polymorphonuclear leukocytes (low power magnification, × 394).

C, large polykaryocyte, breaking up, surrounded and invaded by accumulating leukocytes (low power magnification, × 394).

D, polykaryocyte, 69 by 57.2 microns, showing a great number of nuclei in a strongly basophilic cytoplasm. This represents dissociation of nuclear and cytoplasmic maturation.

of the "functional area" observed in normal granulopoiesis in every detail, with the only difference that the formation of the azurophilic granules is suppressed. The granules are entirely absent in most of the cells. Only exceptionally an adult megakaryocyte may show some scarce granules of faintest stain. The hyalinization does not attack the cell body in a diffuse manner, nor in multiple foci. It involves the cell body by concentric growth, leaving just a narrow rim, in a manner identical with that in which the normal area of granulation tapers off toward the periphery, leaving only a narrow granule-free border zone. This complete parallelism reveals the nature of the hyalinization process. A functional disorder, not a degenerative transformation, accounts for the features of the pathologic megakaryocytes and their development. The cell begins its functional activity, but the granules fail to materialize. The disturbance differs essentially also from the aforementioned dissociation of nuclear and cytoplasmic maturation, in which the structural and staining properties of the promegakaryocytes are preserved but no trace of a functional area can be detected. In dissociation, therefore, the cell does not enter at all into the functional phase. Dissociation never becomes a systemic disturbance; it remains restricted to a few cells and can be found in any condition of the marrow, whereas the process described here affects the majority of the megakaryocytes and seems to be an exceptional occurrence. The dissociation impresses one as the effect of local accessory factors as suggested by me [32] for the cellular gigantism of erythroblasts, whereas the alteration of the "functional area" described here represents a systemic disorder.

The question as to how frequently this disorder occurs in essential thrombopenic purpura is of great importance. The paucity of cases with definite changes of the megakaryocytes as compared with the great numbers of the type with normal granulopoiesis was emphasized earlier in this study. Among the reports of the former appear a few in which hyaline megakaryocytes are mentioned. Seeliger [2] described 2 cases with such megakaryocytes. In the one 61 per cent, in the other 87 per cent, of the megakaryocytes had a hyaline cytoplasm, deprived of granulations. Only in a few of them were some granules scattered through the cytoplasm. Normal granulation was seen in but a small percentage. In some of the cells the cytoplasm presented a streaky, filamentous structure. The nuclei were polymorphous or broken into pieces. Frank's [17] identical description is probably based on Seeliger's material. The colored picture of a megakaryocyte from Seeliger's slides reproduced by Frank, also Seeliger's [2] illustrations in black, show no resemblance to the hyalinization observed by me. Frank's [17] picture (fig. 5, *6*, on page 364) gives more the impression of indistinctly stained

31. Footnote deleted by the author.

32. Schwarz, E.: Am. J. Anat. **79**:75, 1946.

granules than that of hyaline cytoplasm. Vacuoles which could be thought to be analogous to a hyalinized "functional area" are not mentioned. Stern and Hartmann [20] also found a filamentous, ungranulated, hyaline cytoplasm in 52 per cent of the megakaryocytes, whereas the other cells carried a few granules only. The nuclei were large, bizarre. Their illustrations show no similarity when compared with the megakaryocytes of the present case. Schmincke [22] found distinct granulation only in a few instances. In the majority the cytoplasm showed an irregular, jagged or fringed outline, forming a badly colored, in places speckled mass, sometimes in full decay (material from necropsy). Limarzi and Schleicher [25] followed the effect of splenectomy on the megakaryocytes in 5 cases. The significant postoperative change was a decrease of the early and a correlative increase of the mature cells. One class of them, termed degenerated, showed hyaline cytoplasm, absence of granules and normal nuclei. The authors claimed that this type disappeared after splenectomy. Their picture (fig. 6, *2*) of such a hyaline cell reminds one of vacuolation and does not resemble the hyaline cells illustrated here. Dameshek and Miller [29] observed inhibited production of platelets or absence of granulation in some more mature megakaryocytes and noted that the production of platelets had been shifted from the more mature to the promegakaryocytes. They recorded in their tables the absence of hyaline cells among the group classified as degenerated. Vacuolation was rather frequent. Only one of their examples (fig. 14) recalls by its shape a large hyaline "functional area," although its contents seem too dense for it to be identified with our findings. Revol [27] and Valentine [30] reported that the number of degenerated megakaryocytes was reduced after splenectomy but did not give a description of the degenerative changes.

Fieschi and Villalobos [26] gave more explicit details of the alterations. They stated that more profound changes of a degenerative or "involutive" type are rarely encountered in Werlhof's disease. Vacuolation is usually present, but to various degrees. These vacuoles are located peripherally in the cells and may unite to form larger cavities, which involve the cytoplasm more and more and press the nucleus toward the periphery. This abnormality was found in only 2 cases, in one of them in a highly conspicuous degree. They interpreted this abnormality as "megakaryotoxicosis" or as degeneration based on a constitutional deficiency (*anormalità originaria*). This description and my own observations have some points in common. Especially, figure 13 of Fieschi and Villalobos resembles my figure 1, *I*. But a principal difference lies in the development, which in the present case consists in concentric growth of the hyaline "functional area" toward the periphery, whereas Fieschi and Villalobos assumed a confluence of peripherally arising vacuoles. The latter process must be regarded,

according to our experience and the data scattered in the literature, as independent of any special morbid condition of the bone marrow. Vacuolated megakaryocytes, early and adult as well, may even be found in normal marrow. From the standpoint of Fieschi and Villalobos the condition described by them would in fact represent but an exaggeration of a normal involutive process. Nevertheless, a doubt of the correctness of that interpretation may be ventured. The hyalinized "functional area" may have been confused with peripheral vacuolation. This error is likely to happen as long as the observer is not familiar with the "functional area" and its development.

One may assume from this survey that the disorder of granulopoiesis represented in the present case is not quite exceptional, that in a few of the occurrences reported by other authors similar changes have been noted. Their true nature has not been recognized because normal granulopoiesis and its development had not yet been analyzed. Therefore, the characteristic features of the process were not emphasized as such and were confounded with degenerative changes. Nevertheless, one should refrain from generalizing and admit that one or the other of the quoted descriptions suggests truly degenerative changes.

The recognition of the hyalinization as a functional disturbance obviously influences the concept of "essential thrombopenic purpura." The type described here certainly stands apart from the common type with preserved granulopoiesis and therefore does not seem to suggest a splenic inhibitory factor. The points in which the hyalinization principally differs from degeneration also make it improbable that a toxic agent is responsible. It is my impression that a deficiency of a substance necessary for granulopoiesis constitutes the basic disorder. This deficiency must concern not just a material which enters into the granules as a chemical building stone but rather catalysts participating in the metabolic process leading to the formation of the granules. Of course, such an interpretation is at present only a working hypothesis.[33] According to the analysis presented here, cases of "essential thrombopenic purpura" may fall into three groups, not directly related to one

33. F. Skelton, E. Shute, H. G. Skinner and R. A. Waud (Science **103**:762, 1946) claim, on the ground of experiments and clinical observations, a surprising antipurpuric action of vitamin E (alpha tocopherol). The synthetic drug ("ephynal acetate") cured and prevented purpura produced in dogs by stilbestrol. Five patients suffering from thrombopenic purpura recovered quickly. Bleeding time, capillary fragility and platelet count returned to normal. Nevertheless, it is improbable that all 5 patients treated with vitamin E had purpura of the rare type described here. Furthermore, since this vitamin is present in nearly all foodstuffs, there is little danger that a chronic deficiency of vitamin E may develop. The beneficial effect of "ephynal acetate" should be considered, therefore, as a special pharmacodynamic action of the high amounts (200 to 400 mg. daily) administered to these patients during a long period.

another: those with intact granulopoiesis, which are the common type; those with functional disturbance of granulopoiesis, and those with degeneration or destruction of megakaryocytes.

SUMMARY

A case of chronic essential thrombopenic purpura with exceptional changes of the megakaryocytes is described. Hyalinization and absence of the azurophilic granulation were the pathologic features.

The changes were followed from their beginning in the megakaryoblast throughout the development of the megakaryocyte. The changes were not due to a degenerative process but corresponded to a functional disturbance of granulopoiesis. The progressive stages of the disorder are in full agreement with those of normal granulopoiesis as it was analyzed in the foregoing paper.

The observed disturbance cannot be ascribed to a splenic factor or to damage caused by a toxin. A probable hypothesis assumes a deficiency of substances necessary for granulopoiesis.

Consequently, "essential" thrombopenic purpura splits into three types, not related to one another: (1) the common type with preserved granulopoiesis and partial influence of a splenic factor; (2) a type of functional failure of granulopoiesis, probably in the nature of a deficiency; (3) a type with degenerative or destructive changes of cytotoxic origin.

PATHOLOGIC ANATOMY OF ACUTE EXPERIMENTAL BERYLLIUM POISONING

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DURING the past five or six years, many new uses have been found for beryllium; as a result, the isolation of this metal from the ore and the production of various beryllium compounds have increased tremendously during this time. Two types of human illness have appeared where workers have been exposed to beryllium compounds: "acute pneumonitis" [1] and "chronic pulmonary granulomatosis" (delayed chemical pneumonitis).[2] This has stimulated considerable interest in the biologic effects of a previously little studied metal. The work to be reported was undertaken in order to define as precisely as possible what tissues or organs may be affected by beryllium salts, what the acute lesions are, and what quantities of beryllium salts are required to produce the lesions. Such information is of importance not only to the experimental pathologist but also to the biochemist who is seeking an explanation of the toxicity of the metal.

The gross and microscopic descriptions to be given have to do mostly with the changes occurring in tissues after inhalation of hydrated beryllium sulfate ($BeSo_4.4H_2O$) and after intravenous and intraperitoneal administration of the substance. Since beryllium sulfate acts locally as an irritant, causing an inflammatory reaction and sometimes necrosis (e. g., that following subcutaneous administration), one must distinguish between the changes resulting from generalized toxicity and the local response (e. g., that which occurs in the lungs when the compound is inhaled). Beryllium sulfate has been used almost exclusively in these studies because it can be obtained in rather pure form and because there is little reason to think that the sulfate ion would modify or mask the reactions of the tissues. The rat has been used for most of this work. In general, tissue changes were approximately the same in all

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From the Department of Radiology, Division of Pharmacology, University of Rochester, School of Medicine and Dentistry.

1. Van Ordstrand, H. S.; Hugh, R.; De Nardi, J. M., and Carmody, M. G.: J. A. M. A. **129**:1084, 1945.

2. Hardy, H. L., and Tabershaw, I. R.: J. Indust. Hyg. & Toxicol. **28**:197, 1946.

the species receiving beryllium sulfate intravenously (rats, rabbits and guinea pigs), although it should be noted that there were considerable differences in the dosages required to kill animals of different species. The intensity and even the location of lesions varied somewhat with the dosage; therefore, some statement concerning toxic levels should be made. For white female rats weighing 175 to 200 Gm. the intravenous $L.D._{50}$ for beryllium sulfate was around 7.2 mg. per kilogram (0.36 mg. per kilogram of beryllium); a few animals have died at 6 mg. per kilogram, and a few have survived at 8 mg. per kilogram.

GROSS OBSERVATIONS

Little was found grossly in the organs of animals dying after intravenous administration of beryllium sulfate. When large doses (30 to 60 mg. per kilogram) were given, petechial hemorrhages were seen on the serous surfaces; there was a considerable increase in pleural fluid, which sometimes was bloody; the spleen was enlarged and deep purple. Following administration of smaller doses, around $L.D._{50}$, none of these changes were seen. Jaundice was observed in all rats dying three days or more after a single intravenous administration of beryllium sulfate.

MICROSCOPIC OBSERVATIONS

Liver.—The most conspicuous pathologic feature seen after a single intravenous administration of beryllium sulfate was necrosis of liver cells. When large doses were given, the necrosis was found within sixteen to eighteen hours; with smaller doses (6 mg. per kilogram), it was not histologically clear until the third day (seventy-two hours). The extent of the necrosis also varied directly with the dosage. Only a few of the rats that received 5 mg. per kilogram have shown hepatic necrosis; all that received 6 mg. per kilogram have shown necrosis. The earliest lesion was observed in the midzonal portion of the hepatic lobule and consisted of a small focus of cells in which the cytoplasm assumed a homogeneous eosinophilic stain and the nuclei became pyknotic (fig. 1). The amount of necrosis varied from a few scattered small foci, each consisting of only a few cells, to almost complete necrosis of the organ, with only a layer or two of viable cells around the portal areas. Usually, there was no inflammatory reaction about these areas of necrosis.

The liquefaction and absorption of the necrotic cells proceeded rather slowly, usually requiring two to three days. During this period (from the third to the sixth day after a single intravenous administration) one saw varying numbers of necrotic cells and areas in which cells had been absorbed, leaving foci of closely packed vascular sinusoids and stroma. These foci consisted of a stroma of reticulum and collagen in which endothelial cells from the sinusoids, phagocytic cells, and a few granulocytes and lymphocytes were present (fig. 2). There seemed to be no increase in collagen and, at least after a single intravenous administration, no perceptible scar has been found. There was no evident injury of the collagen or the reticulum.

The regeneration of the liver, as indicated by an increased number of mitoses, usually became evident on the fifth to sixth day after a single administration of the beryllium sulfate. This proceeded quite rapidly, and by two weeks, and usually in less time, the liver had a normal microscopic appearance.

The behavior of fat, as stained by sudan IV, is worth mentioning. The necrotic liver cells contained a moderate amount of finely divided brownish-staining fat,

which disappeared when the cells disappeared. Immediately adjacent to the foci of necrosis, the liver cells, which appeared normal, with an ordinary hematoxylin and eosin stain, contained a large amount of coarse deeply staining droplets. These remained during the entire period of liver regeneration and disappeared sometime between the eighth and the fourteenth day.

In regenerating livers, after this type of injury, the irregular, nodular hyperplastic lobules, the large, multinucleated hepatic cells and the heavy cords of hepatic cells have not been observed. Moreover, we have not observed thrombi of bile canaliculi, an excess of bile in the interlobular bile ducts or the inflammatory reaction of the portal areas so frequently observed in other types of hepatic injury.

Kidney.—The histologic renal changes were not nearly as extensive or conspicuous as those of the liver; however, they occurred at a dose somewhat lower than that which produced hepatic necrosis. Thus, 6 mg. per kilogram of beryllium sulfate produced definite and constant necrosis of the liver, whereas 5 mg. per kilogram produced only minimal necrosis of the liver in an occasional rat; however, at 5 mg. per kilogram most rats showed definite renal changes on the fourth or the fifth day. Also, the amount of renal damage has not seemed to be proportional to dosage. This may, perhaps, be due to the fact that the excretion of beryllium cannot exceed a certain maximum concentration. It might be noted also that the most severe renal changes have been observed in rabbits which inhaled beryllium sulfate as a dust.

The renal changes seen were almost entirely limited to the distal portion of the proximal convoluted tubule; however, in some few animals, changes have been observed to a lesser extent throughout the entire proximal convoluted tubule. In the case of the rabbits, some changes were seen in the descending loops of Henle, and there was considerable dilatation of the collecting tubules. The lesion consisted in necrosis of variable numbers of epithelial cells. The first change observed was a change in staining reaction from the ordinary finely stippled pink of the cytoplasm (eosin stain) to a deeper homogeneous reddish color. The nuclei of such cells became pyknotic. Such cells could be found still lining the tubule and apparently resting on the basement membrane. They were soon desquamated and were found in the lumens of the tubules distal to their origin. Epithelial casts were occasionally seen (fig. 3).

Hemopoietic System.—When beryllium sulfate was administered to rats in large doses (30 to 60 mg. per kilogram) the spleen became enlarged, engorged and purple. Microscopically, the sinusoids of such a spleen were extensively engorged with blood and fluid, and there was a marked decrease in the usual number of nucleated cells. In such an organ one saw nuclear degenerative changes. The lymphocytes showed clumping of nuclear chromatin at the periphery, and this was probably followed by karyorrhexis, since abundant amounts of nuclear debris could be seen throughout the red pulp. The malphighian corpuscles seemed to contain many fewer cells, and no active germinal centers were observed. In animals receiving doses of beryllium sulfate around the $L.D._{80}$ or less, these changes were not so marked.

The bone marrow showed granulocytic hyperplasia. With the higher doses there were degenerative changes in the nuclei. The lymph nodes showed little detectable change, except that with high doses some degenerative changes were noted in the nuclei of the lymphocytes. The effects of beryllium sulfate on the hemopoietic system can be studied better in the peripheral blood counts. The changes in these will be presented in a subsequent publication.

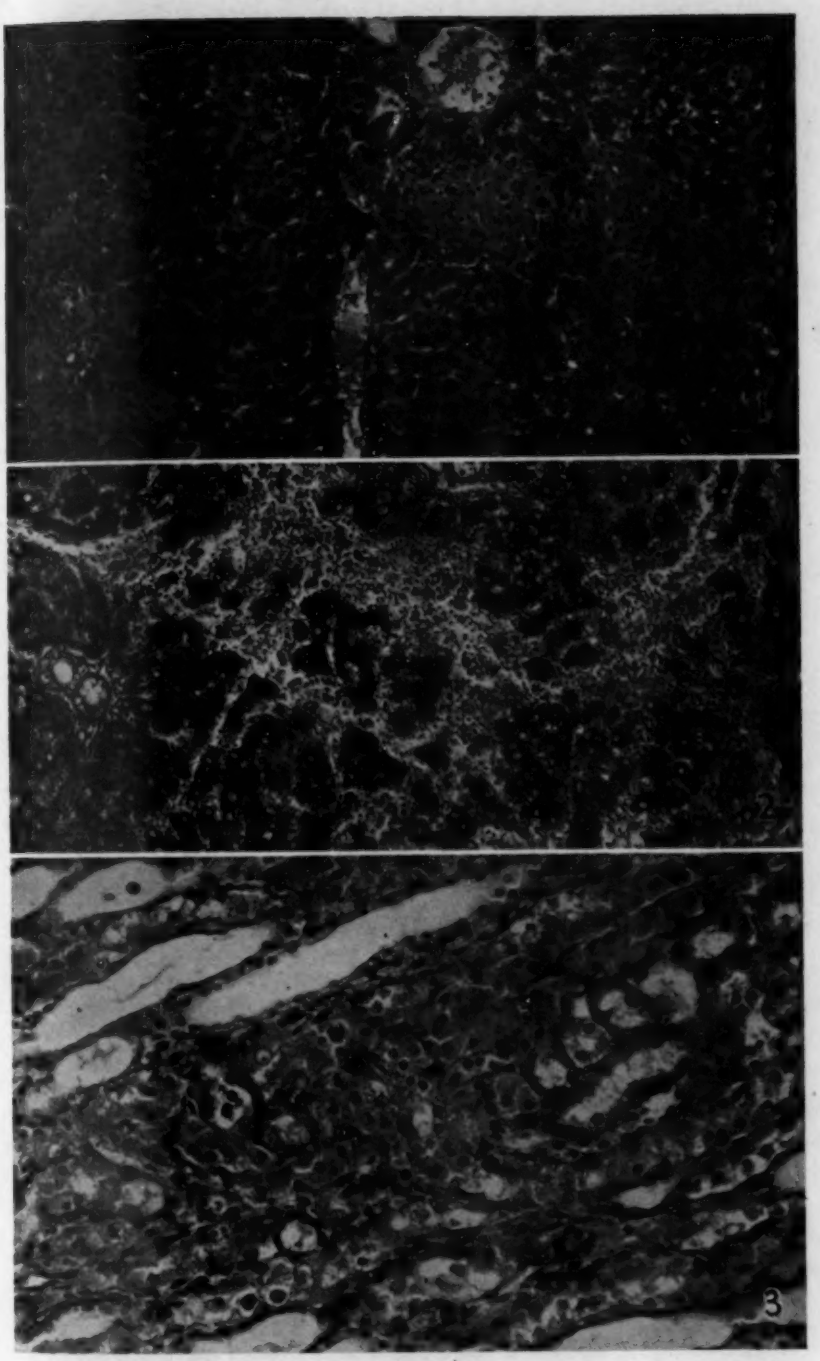

Fig. 1.—Liver of a rat killed three days after receiving beryllium sulfate intravenously—6 mg. per kilogram. The liver shows small midzonal areas of necrosis, the earliest change noted at this dosage. × 120.

Fig. 2.—Liver of a rat killed eight days after receiving beryllium sulfate intravenously—6 mg. per kilogram. It shows the hepatic structure after the necrotic hepatic cells have disappeared. Wilder reticulum stain; × 100.

Fig. 3.—Kidney of a rabbit killed after being exposed six hours daily for eleven days to an atmosphere containing beryllium sulfate in a concentration of 88 mg. per cubic meter. It shows necrosis and desquamation of tubular epithelial cells. × 120.

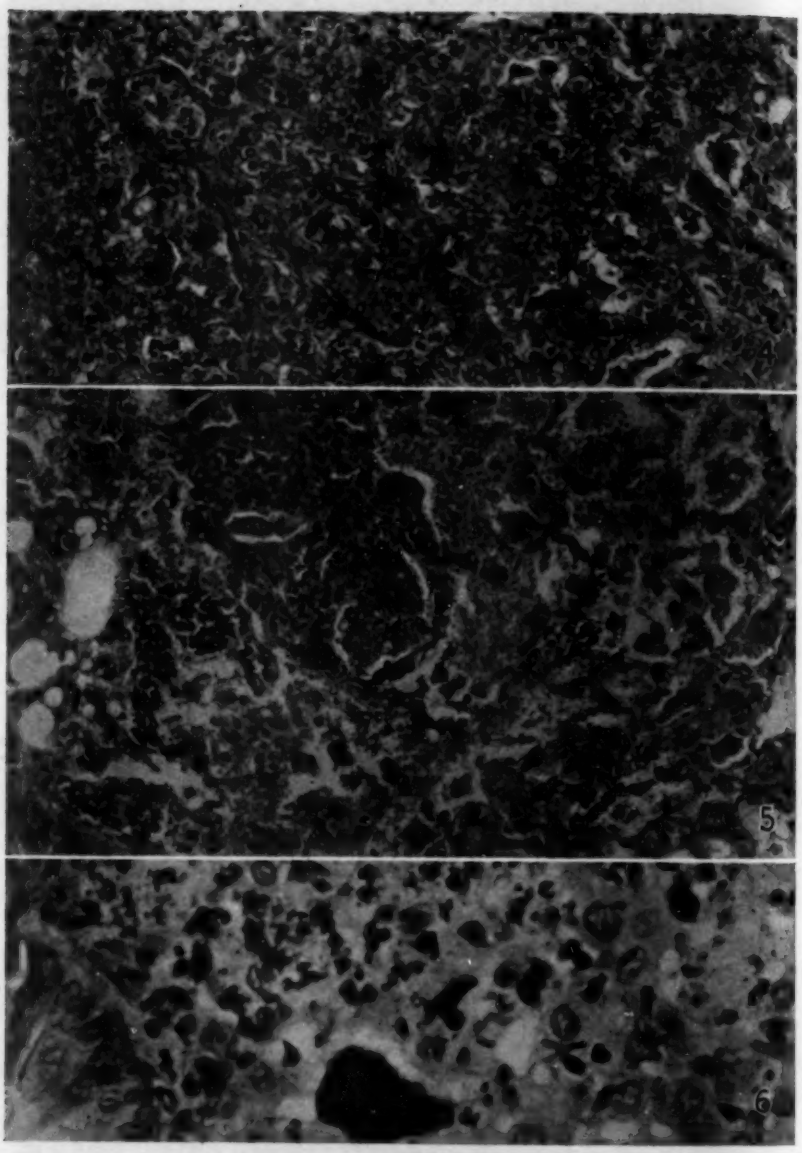

Fig. 4.—Lung of a guinea pig exposed to the same atmospheric conditions as the rabbit of figure 3. It shows the type of pulmonary reaction occurring in this species. × 120.

Fig. 5.—Lung of a rabbit exposed to the same atmospheric conditions as the rabbit of figure 3. It shows a deeply stained coagulated exudate containing nuclear debris. × 120.

Fig. 6.—Lung of a rat killed seven days after receiving beryllium metal dust intratracheally—100 mg. per kilogram. It shows an exudate containing peculiar blue bodies. × 400.

Local Effects of Beryllium Sulfate on Tissues.—We have had opportunity to study this problem only on the eyes and the respiratory tract in animals (rats, mice, guinea pigs, hamsters, rabbits, and dogs) which had inhaled a beryllium sulfate dust six hours daily for eleven days. The median particle size was approximately 4 microns, and the concentration of the dust was approximately 88 mg. per cubic meter of inhaled air.

The guinea pigs and the dogs showed the most extensive and frequent ocular changes. These consisted in conjunctivitis with the usual microscopic picture of an acute or an organizing inflammation. In many of the animals a rather severe degree of keratitis occurred, consisting in vascularization of the cornea, development of granulation tissue and frequently rather marked ulceration. In some animals the anterior chamber contained a purulent exudate, which was associated with mild uveitis.

The lungs of all the animals showed histologic changes; in some species (e.g., the guinea pig) the lesions were few and not diffuse; in others (e.g., the rabbit) the lesions were more widespread and frequently involved most of one or more lobes. An inflammatory exudate has been found in the lumens of the terminal bronchi in all species. There was a coagulated exudate in which nuclear debris was found—the latter probably arose from disintegrating granulocytes and bronchial epithelial cells (figs. 4 and 5). Superficial ulceration of bronchial epithelium was frequently present, which was accompanied by bronchial epithelial proliferation. Some atelectasis, usually involving only isolated lobules, was seen. In some species there was a fairly marked pulmonary edema. The granulocytic cellular exudate so frequently found in other inflammatory processes has not been particularly prominent: The alveolar sacs usually contained a few large phagocytic cells, and the bronchi contained a few granulocytes. No pulmonary fibrosis has been observed in any of the animals studied thus far. However, occasionally fibroblasts have been seen growing into the lumens of the bronchi.

A number of animals receiving beryllium compounds or beryllium metal intratracheally or intraperitoneally have shown peculiar blue-staining structures (fig. 6). The process by which these bodies originate, develop and disappear is not understood.

SUMMARY

The lesions occurring in various organs after administration of beryllium sulfate have been described. These were focal necrosis of the liver, tubular epithelial necrosis of the kidney and degenerative changes of the hemopoietic system. The pulmonary lesions occurring after inhalation of beryllium sulfate dust have been described.

INTRACELLULAR INCLUSION BODIES IN MILD DISEASE OF THE RESPIRATORY TRACT

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ALTHOUGH the more easily visible nasal and pharyngeal mucous membrane has been examined grossly in mild infections of the respiratory tract, few histologic studies have been made. This study was undertaken to determine whether the microscopic appearance of the pharyngeal exudate is in any way distinctive in such infections. Clinicians and pathologists have focused their attention chiefly on the more striking lesions in the lower part of the respiratory tract and have neglected to describe the pathologic changes in the upper air passages. In 20 or more cases in which death was caused by viral pneumonia the necropsy reports give no description of the part of the respiratory tract above the trachea.[1]

Broadhurst and her associates,[2] in 1936, observed cytoplasmic inclusion bodies in the pharyngeal epithelial cells of 76 per cent of persons with mild infections of the respiratory tract, but also found them in the corresponding cells of 8 per cent of healthy persons. Intranuclear inclusions were subsequently noted in epithelial cells of the upper respiratory tracts of infants with pneumonia following whooping cough,[3] and both cytoplasmic and nuclear inclusions in the corresponding cells of infants with "giant cell" pneumonia.[4] Adams and co-workers[5] found cytoplasmic inclusion bodies in the pharyngeal epithelial cells of infants during an epidemic of viral pneumonia. Adams and other

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1. Reimann, H. A.: Medicine **26**:167, 1947.

2. Broadhurst, J.; Liming, R. M.; Maclean, M. E., and Taylor, I.: J. Infect. Dis. **58**:134, 1936.

3. Goodpasture, E. W.; Auerbach, S. H.; Swenson, H. S., and Cotter, E. F.: Am. J. Dis. Child. **57**:997, 1939.

4. Pinkerton, H.; Smiley, W. L., and Anderson, W. A. D.: Am. J. Path. **21**:1, 1945.

5. Adams, J. M.; Green, R. G.; Evans, C. A., and Beach, N.: J. Pediat. **20**:405, 1942.

co-workers [6] studied the acutely inflamed pharynx in influenza and reported the most characteristic single feature to be a mononuclear exudate, which is consistent with similar changes in other infectious processes caused by filterable viruses and is found in a few adult patients with viral pneumonia.

Intranuclear or cytoplasmic inclusion bodies are frequently associated with infections caused by viruses, and to this extent their presence in the epithelium of the respiratory tract adds further evidence that a virus or viruses are the cause of certain as yet undifferentiated infections of the respiratory tract. Cellular inclusions are also present, however, in persons without obvious infection, and may be induced to appear by chemical or other irritants. It is uncertain whether the inclusions are aggregates of virus particles, as believed by some, or whether, more likely, they result from viral or chemical injury of the cytoplasm.

A further study of the problem was made during an epidemic of mild infection of the respiratory tract in the winter of 1947.

METHODS

Exudate was obtained from the oropharynx with a sterile cotton swab and transferred to a glass slide by gently rolling the applicator over it.[5] After drying, the preparation was stained with Seller's basic fuchsin–methylene blue method. When sputum was available, similar preparations were made of it. In most instances daily examinations were made. The exudate was also cultivated on blood agar in order to determine the presence or the absence of the hemolytic streptococcus as a cause of infection.

RESULTS

Normal Subjects.—Over a three month period, material was taken from the pharynxes of 90 normal persons on 120 occasions and examined. The majority of the specimens studied were from 57 persons who were apparently well. Inclusions were found in 7 instances. The proportion of cells containing inclusions varied from 1 to 16 per cent.

Subjects with the Common Cold.—Of the 33 persons with mild symptoms characteristic of a "cold" or of slight sore throat with minimal to moderate injection of the blood supply of the pharynx but without visible exudate, 20 were found to have cytoplasmic inclusions in epithelial cells of the pharynx. The proportion of cells containing inclusions varied from 2 to 28 per cent. In the initial stage of infection the smears were usually relatively free of bacteria and inflammatory cells, but twenty-four to forty-eight hours later many bacteria and polymorphonuclear leukocytes were seen. Thirteen persons had smears

6. Adams, J. M.; Pennoyer, M. M., and Whiting, A. M.: Am. J. Dis. Child. **71**:162, 1946.

that revealed no inclusions but contained bacteria and polymorphonuclear leukocytes, suggesting that they were studied late in the infection, after the inclusions had disappeared and bacteria and purulent exudate were present. This was substantiated in most instances by the history, which revealed that the symptoms had been present for more than twenty-four hours.

In 10 instances the subject was studied before, during and after a "cold with mild sore throat." The course of the disease was reflected in the changing character of the smears. Before infection only a few bacteria and epithelial cells were seen. During the first twenty-four hours of disease there was a great increase in the number of shed epithelial cells and in the number of cytoplasmic inclusions in them. After another twenty-four hours the inclusion bodies were replaced, as before, by bacteria and polymorphonuclear cells. In a few instances in which epithelial cells contained an occasional inclusion in the predisease period, a great increase in the number of inclusions occurred during the early inflammatory stage.

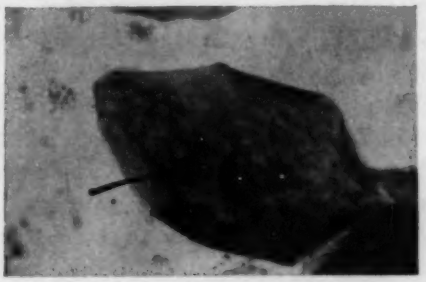
Patients Admitted to the Hospital.—Ten persons were studied who were sick enough with acute nonbacterial pharyngitis to be admitted to the hospital. From clinical and laboratory evidence the infection appeared similar to, or identical with, that reported by the Army Commission on Acute Respiratory Diseases.[7] The onset was usually gradual over a period of twelve to twenty-four hours. Slight sore throat was the prominent symptom, but headache, chilliness, malaise, photophobia, hoarseness and cough were present. In some instances, there was substernal pain, and the course lasted from forty-eight to seventy-two hours. The patients appeared to be mildly sick. The pharynx was diffusely reddened, and the lymph follicles were enlarged. In half the cases a small amount of gray exudate covered the inflamed membrane. The tonsils when present showed congestion of blood vessels. The regional lymph nodes apparently were not affected. The chest was normal. The leukocyte count was normal. Bacteriologic studies did not reveal obvious pathogenic bacteria, and roentgenograms of the chest showed no changes.

Studies of the exudate, which was stained in the usual manner, were made daily. The findings were essentially the same as those reported by Adams in influenza A except for the presence of inclusion bodies. Sheets of desquamated epithelium were present in all cases, and in all but 1 case cytoplasmic inclusions like those described before

7. Commission on Acute Respiratory Diseases, J. A. M. A. **125**:1163, 1944; Am. J. Pub. Health **35**:675, 1945; J. A. M. A. **133**:588, 1947.

were present (figure). The percentage of cells containing them varied from 10 to 30, with an average of 18. Inclusions persisted from forty-eight to seventy-two hours during the infection. Also present was an exudate composed almost entirely of monocytes. A few plasma cells and polymorphonuclear cells were present. In 1 case, smears from the pharynx contained chiefly exfoliated epithelial cells, but the sputum contained mostly mononuclear cells, many bronchial and alveolar epithelial cells, 17 per cent of which contained inclusions.

As in the patients with mild colds, inclusion bodies and mononuclear cells were replaced after forty-eight to seventy-two hours with polymorphonuclear cells and many bacteria, suggesting bacterial invasion, as noted in Broadhurst's studies.[2] Inclusions were rarely found when bacteria were numerous. Yet in only 1 case was clinical evidence of bacterial invasion present in the form of acute left maxillary sinusitis



Epithelial cell of the pharynx containing three cytoplasmic inclusion bodies; × 900.

with pus containing only Staphylococcus aureus. Although histologic evidence suggests that, as a rule, bacterial invasion occurs, clinical or other evidence of such invasion is exceptional. It is possible, therefore, that the word "invasion" is inappropriate in most instances. The polymorphonuclear exudate may represent only the terminal reaction to previous injury, and the heavy growth of bacteria may signify merely that conditions are conducive to their growth. In rare instances actual invasion occurs.

THE INCLUSION BODIES

With Seller's stain, the cytoplasm of the cells stained pink and the nuclei blue. The inclusions usually appeared as magenta-colored bodies and were homogeneous in appearance, although often they were bright red and stippled, giving the impression that they were an aggregate of particles. In size the bodies varied greatly and were between 0.5 and 1.5 microns in diameter (figure). Variation in size was noted not

only in the same preparation but within the same cell. In the majority of cases the inclusions were round, although in a few instances oval forms were seen. Occasionally there was a transparent halo about the inclusion bodies. No orderly grouping of inclusion bodies was noted. They were scattered throughout the cytoplasm and were distinctly separated from one another. In preparations containing few inclusion bodies it was unusual to find an epithelial cell containing more than one inclusion body. When many were present, from 8 to 12 inclusions might be found within a single epithelial cell although 3 to 4 to a cell was the usual finding. The epithelial cells containing the inclusions frequently appeared abnormal. The cytoplasm showed disintegration, only a small rim of it being left around the nucleus, in which an inclusion or two was still embedded. When many inclusions were present, extracellular forms were also generally seen. In such preparations, disintegrated epithelial cells were present in large numbers, and the extracellular forms may have represented bodies released by disintegrated epithelial cells. One could not help but note the paucity of bacteria in smears when many inclusion bodies were present. There appeared to be a reciprocal relationship between number of inclusion bodies and number of bacteria. In a daily study of smears of material taken from the pharynx of the same subject it was noted without exception that as the inclusion bodies decreased in number bacteria became more prominent. This is in agreement with the observations of Broadhurst and her associates,[2] who also noted the apparent incompatibility between bacteria and inclusion bodies, and of Dochez and his co-workers,[8] who reported that in apes the significant effect of infection with a filtrable agent obtained from persons with the common cold was an increase of the activity of potential pathogens present in the upper respiratory tract.

SUMMARY

Histologic studies of the pharyngeal mucous membrane during mild infections of the respiratory tract confirm previous observations as to the presence of cytoplasmic inclusion bodies and a mononuclear cell exudate during the early stage in the majority of cases. Inclusions were also found in 12 per cent of smears of material from normal pharynxes. The significance of the presence of the bodies is not certain, but they are generally believed to represent injury of the cytoplasm caused by a filtrable virus or other irritant. To this extent their presence adds more evidence to favor the viral origin of these undiffer-

8. Shibley, G. S.; Mills, K. C., and Dochez, A. R.: J. A. M. A. **95**:1553. 1930.

entiated infections. It is questionable whether the later appearance of the polymorphonuclear cell exudate and bacteria always indicates bacterial invasion rather than the nonspecific reaction to inflammation and the provision of conditions favorable to bacterial growth.

The regular increase in the number of bacteria present is cited by many to justify the use of sulfonamide compounds or of antibiotics in the treatment of the condition or to prevent metastatic invasion elsewhere. In view of the imperfect knowledge of the matter, the value of such therapy is highly uncertain.

Case Reports

AMNIOTIC FLUID EMBOLISM

Report of a Fatal Case

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PULMONARY EMBOLISM occurring in the parturient or the puerperal state is most frequently due to material of a thrombotic nature. Emboli of air, fat or abortifacient paste found in pregnant women are of medicolegal importance. A uterine sinusoid–pulmonary capillary pathway has been frequently demonstrated by the finding of cytotrophoblastic elements or decidual cells in the pulmonary circulatory bed in women dying in the first few days of the puerperium from other causes.

Present knowledge of sudden obscure deaths during labor and the puerperium was advanced by Steiner and Lushbaugh[1] in 1941 with their demonstration of pulmonary embolization due to the presence of particulate constituents of amniotic fluid. These authors, on the basis of the 10 cases which they reported, stressed that many fatal conditions diagnosed clinically as obstetric shock, acute pulmonary edema, premature separation of the placenta, postpartum hemorrhage or thrombotic pulmonary embolism were probably fatalities due to amniotic fluid embolism.

Gross and Benz,[2] Hemmings[3] and Burt,[4] in their recent reports concerning this entity, reveal that 15 proved cases of amniotic fluid embolism have been recorded in the literature.

REPORT OF CASE

I. M., a 30 year old primipara, was admitted to Toronto East General Hospital in labor. Her pregnancy had been uneventful, and labor had started spontaneously. On admission her temperature and her pulse and respiratory rates were normal, and pains were coming every five minutes. The membranes were intact, and slight show was present. The blood pressure was 140 systolic and 80 diastolic, and the fetal heart rate was 130. On admission, 1 ampule (1 mg.) of "synkamin" (4-amino-2-methyl-1-naphthol hydrochloride), a synthetic vitamin K, was given, vaginal preparation was carried out and a soap suds enema was administered.

From the Department of Pathology, Toronto East General Hospital.

1. Steiner, P. E., and Lushbaugh, C. C.: J. A. M. A. **117**:1245, 1941. Lushbaugh, C. C., and Steiner, P. E.: Am. J. Obst. **43**:833, 1942.

2. Gross, P., and Benz, E. J.: Surg., Gynec. & Obst. **85**:315, 1947.

3. Hemmings, C. T.: Am. J. Obst. **53**:303, 1947.

4. Burt, R. C.: Read before the Clinical Pathological Society of Pittsburgh, May 15, 1946; cited by Gross and Benz.[2]

One hour after admission 100 Gm. of meperidine hydrochloride ("demerol hydrochloride") and 1½ grains (0.097 Gm.) of sodium propylmethylcarbimylallylbarbiturate ("seconal sodium") were given. One hour later the membranes had ruptured and the caput was presenting. The patient climbed onto a stretcher by herself to be taken to the case room and then suddenly became cyanotic and short of breath. Despite administration of a 25 per cent nikethamide solution and oxygen via an airway the patient died in fifteen minutes.

Autopsy (seven hours after death).—The body was that of a 30 year old woman with a retained full term pregnancy. Patchy cyanosis of the face was present. Recent petechial abrasions were present in the larynx, presumably caused by the passing of the airway in the attempt at resuscitation.

The lungs were grossly normal, and each weighed 340 Gm. The spleen weighed 210 Gm.; this amount of enlargement is common in pregnancy. Minimal hydronephrosis and hydroureter were present on the right side, due to the ureter having been compressed by the gravid uterus. The uterus was normal for its physiologic state. The remainder of the organs were of average weight and grossly normal. Patency of the foramen ovale was not demonstrated at the postmortem examination.

The fetus weighed 2,735 Gm., and apart from atelectasis and petechiae of the pericardium there were no abnormal findings. The placenta was still attached to the fundus and free of any gross defects. The amniotic fluid that remained contained little meconium. The cervix was completely effaced and its inner surface abraded in the region where the membranes had ruptured. The membranes were otherwise intact and completely lined the uterine cavity.

Microscopic Examination.—All tissues except the brain were fixed in Zenker's solution. The brain was fixed in a mixture of a 4 per cent formaldehyde solution and sodium chloride solution. Paraffin sections were stained with hematoxylin and eosin and Mallory's aniline blue. Frozen sections of the lung were stained with sudan IV.

Lung: All sections examined revealed throughout most of the pulmonary arterioles and capillaries a varying quantity of the constituents of amniotic fluid. Epithelial squamae were the dominant members. These were of a distorted or twisted contour and basophilic in tinctorial reaction. Many of these embolic foreign squamae were entangled with, or boxed in by, gatherings of neutrophilic polymorphonuclear leukocytes. Mucin was present in some of the larger pulmonary arterial vessels. The stringy mucin had trapped many neutrophilic granulocytes. Eosinophilic granular debris was found in some of the larger pulmonary channels. Sudanophilic granules and droplets were demonstrated in the capillaries. The neutrophilic polymorphonuclear leukocytes were all within vessels; none were found emigrating through the wall. Aggregates of red cells were frequently observed in the vessels containing the embolic constituents. Lanugo hair was not identified in the vascular channels of the lungs. The pulmonary veins were relatively bloodless, while the arterial pathway was congested. No endothelial desquamation of the vessel linings was detected. There was no edema of the lung or any other parenchymal change (figs. 1 and 2).

Heart: A number of minute capillary and arteriolar channels showed the occasional basophilic embolic squamae filling the lumens of the vessels. Eosinophilic granular debris was noted in some of the larger channels. There were no lesions in the myocardium (fig. 3).

Uterus: Numerous sections were taken throughout the abraded spongy cervical portion of the uterus. Many of the uterine sinusoids were filled with aggregates

of red blood cells and in addition showed intraluminal epithelial squamae and mucin. A few of the sinusoids showed a rather heavy deposit of these derivatives of the contents of the amniotic sac. The endothelial lining was intact in all the vessels studied (fig. 4).

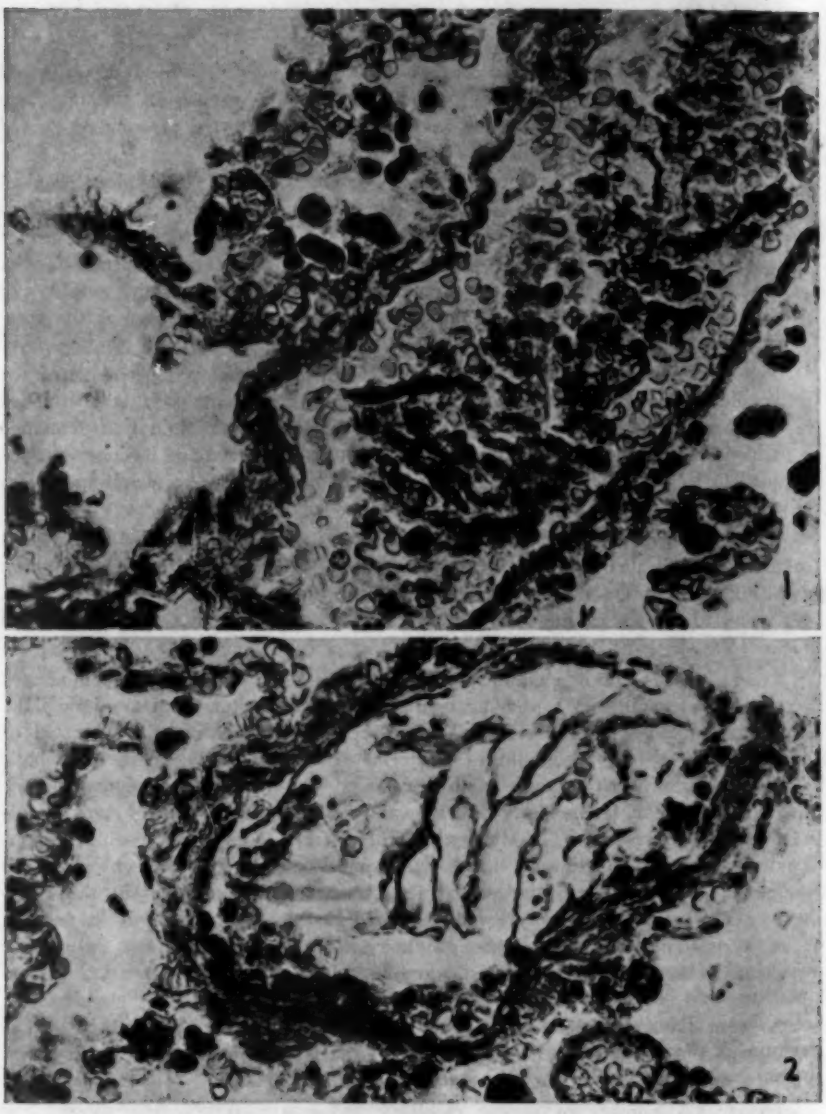

Fig. 1.—Lung with leukocytes mobilized around an individual epithelial squama within a blood vessel; × 480.

Fig. 2.—Lung with embolic strings of mucin within a small artery; × 495.

Sections of brain, spleen, pancreas, gastrointestinal tract, adrenal glands, kidneys, bone marrow, fallopian tubes and ovaries were not remarkable.

COMMENT

This catastrophe of amniotic fluid embolism is ushered in frequently with violent labor pains, chills and intense shortness of breath, followed by cyanosis and profound shock. Death is sudden. At autopsy there are no gross abnormalities. Histopathologic examination is diagnostic.

In this case, microscopic examination of the vascular channels of uterus, lung and heart revealed embolic constituents derived from the contents of the amniotic sac. The dominant embolic elements were the basophilic epithelial squamae, acidophilic granular debris of vernix

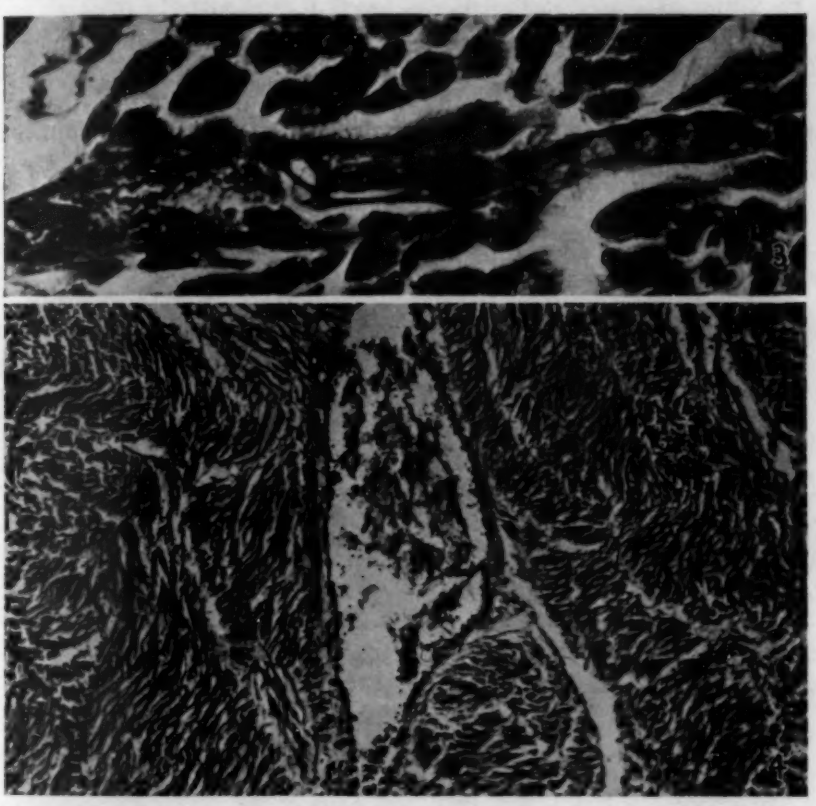

Fig. 3.—Heart with a single distorted angular squama and granular material in a capillary; × 600.

Fig. 4.—Uterus showing stringy mucin and squamae within a uterine sinusoid; × 90.

caseosa origin and mucin. The origin of the mucin, whether of uterine gland or meconium, was not determined. It was noted that there was stratification of leukocytes around these embolic particulate constituents within many of the pulmonary vessels. This "combing out" phenomenon described by Gross and Benz had apparently occurred within a period of fifteen minutes. These polymorphonuclear leukocytes were of marrow-blood origin and not tissual. The adherence of the leukocytes to the

squamae and mucin may represent an interference with the surface tension interface of the blood or "stickiness" of the embolic constituents. Intralumina! aggregation of erythrocytes was another noteworthy histopathologic feature. The pulmonary arterioles and capillaries were congested and showed frequent specific embolic particles; in contrast, the pulmonary veins were relatively empty.

These amniotic contents probably entered the uterine venous pathway through the abraded surfaces of the lower uterine segment at the time the patient climbed onto the stretcher.

The sudden death of the patient occurring within a period of ten to fifteen minutes was due to the intravascular entrance of these foreign elements resulting in embolization of lung and heart or a fatal anaphylactoid reaction.

SUMMARY

A case of fatal amniotic fluid embolism is reported. Constituents of the amniotic fluid were present in coronary vascular channels.

HAMARTOMA OF THE SPLEEN

Report of Three Surgical Cases

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ALBRECHT [1] in 1904 proposed the term "hamartoma" for certain hemangiomas of the liver. He applied the designation to tumor-like malformations consisting of an abnormal mixture of normal components of an organ. Such abnormality, he stated, could be one of quantity, arrangement, degree of differentiation or all three.

Since Albrecht's original description, many authors have utilized the concept of hamartoma to account for lesions found in widely varying situations. For example, Turner [2] in 1937 reported a case of arteriovenous hamartoma of the brain. Clarke [3] in 1940 and Mitchell and Angrist [4] in 1943 reported 8 cases and 24 cases, respectively, of myoepithelial hamartoma of the gastrointestinal tract. Moolten [5] in 1942 called attention to probable examples of disseminated hamartoma, such as von Recklinghausen's disease (neurofibromatosis), Lindau's disease (combined hemangioma of the cerebellum and the retina), Osler-Vaquez disease (splenomegalic polycythemia, erythremia) or heredofamilial angiomatosis of the skin and the mucosa, encephalotrigeminal angiomatosis, congenital polycystic disease of liver, kidneys and pancreas, and, finally, tuberous sclerosis. Stamm and Tauber [6] in 1945 reported a case of hamartoma of the tongue. The lung has been recognized as the site of hamartoma, as several reports have attested. One such report, that of McDonald, Harrington and Clagett,[7] included 23 cases of pulmonary tumors, all of which the authors were able to classify as cases of hamartoma.

From the Division of Surgical Pathology, Mayo Clinic.

1. Albrecht: Verhandl. d. deutsch. path. Gesellsch. **7**:153, 1904.
2. Turner, O. A.: Arch. Path. **24**:223, 1937.
3. Clarke, B. E.: Arch. Path. **30**:143, 1940.
4. Mitchell, N., and Angrist, A.: Ann. Int. Med. **19**:952, 1943.
5. Moolten, S. E.: Arch. Int. Med. **69**:589, 1942.
6. Stamm, C., and Tauber, R.: Laryngoscope **55**:140, 1945.
7. McDonald, J. R.; Harrington, S. W., and Clagett, O. T.: J. Thoracic Surg. **14**:128, 1945.

A reasonably complete search of the literature has indicated that examples of hamartoma of the spleen are not common. A few cases have been reported in which the term "splenoma" has been used to designate this condition.

In 1914 von Falkowski [8] presented a case of an infant who had mesenchymal hamartoma of the liver and the spleen associated with multiple angioma of the skin. The spleen contained scattered nodules both on the surface and throughout the organ. These nodules were sharply demarcated, round and stony hard. Microscopic examination revealed them to be composed of abundant ramifying wide and narrow vessels, between which was a fibrous matrix. In places the vessels appeared as narrow capillary-like spaces, and elsewhere, as wide irregular serpiginous spaces. Whatever their size and form, the spaces were lined by a single layer of endothelial cells or were filled with solid masses of these cells. These nodules seemed to represent a benign condition and their existence was explained on the basis of failure of the embryonal mesenchyme to differentiate into endothelium, supporting tissue and blood elements. Because of the multiplicity of organs involved, von Falkowski considered the condition to be an expression of a systemic disease of the embryonal mesenchyme, resulting in tissue malformation.

Mordasini [9] in 1937 reported 6 cases, in 5 of which a single nodule was present in the spleen and in one multiple nodules. The nodules in the 6 cases varied in size from 1 mm. to 11 cm. in diameter. In 5 of the 6 cases the lesion was an incidental finding at necropsy. In the sixth case the lesion was found in a spleen removed surgically. In 5 of the cases the nodule lay directly under the capsule of the spleen, and in 1 case it was situated in the depths of the parenchyma. The composition of the nodules was practically the same in all instances. They contained varying mixtures of tissues normally found in the spleen. The cellular detail varied from place to place. Many reticular cells and free pulp cells were found. Other cells were lymphocytes, neutrophilic leukocytes, eosinophils, occasional plasma cells, and in some lesions myeloblasts, myelocytes and megakaryocytes.

Mordasini summarized the varying structural patterns as follows: an overabundance of follicles in some cases, principally pulp tissue in others, and in still other cases a prominent connective tissue stroma. Because of these abnormal mixtures of tissue elements, it was concluded that the lesions were hamartomatous.

In 1942 Sweet and Warren [10] recorded the following case:

A 59 year old woman complained of swelling of the abdomen, which she had first noticed eight months before. She had lost 17 pounds (7.7 Kg.) in the eight months.

8. von Falkowski, A.: Beitr. z. path. Anat. u. z. allg. Path. **57**:385, 1914.
9. Mordasini, E.: Virchows Arch. f. path. Anat. **298**:594, 1937.
10. Sweet, R. H., and Warren, S.: New England J. Med. **226**:757, 1942.

With the discovery that she had a palpable abdominal mass on the left side, which was outlined at roentgenologic examination, a preoperative diagnosis of cyst of the spleen was made. The spleen, which weighed 1,120 Gm., was removed. On section a well demarcated bulbous region, which was not separated from the remaining normal splenic tissue by a clearly defined capsule, was found in the lower two thirds of the specimen. Microscopic examination revealed no remarkable change in the splenic tissue proper. Within the bulbous mass, however, there was an irregular increase of fibrous stroma with loss of pulp. The structure of trabeculae, sinusoids and malpighian follicles was fairly well maintained. This tumor, which was regarded as a hamartoma, was clearly separated from the remaining splenic tissue by an incomplete pseudocapsule made up of compressed trabeculae.

REPORT OF CASES

In a study of the splenic neoplasms removed surgically at the Mayo Clinic from 1914 through September 1946, 3 cases of hamartoma were encountered.

CASE 1.—A woman 38 years old registered at the clinic on Sept. 13, 1945 because of symptoms referable to the gastrointestinal tract. Symptoms had been present for many years and consisted mainly of flatulence, bloating and vague abdominal cramps. The past history indicated that she had had malaria in 1930, had undergone removal of a cyst of the left breast in 1940 and had injured the left knee in an automobile accident in 1940.

Physical examination gave entirely negative results. The concentration of hemoglobin and the erythrocyte, leukocyte and differential blood counts were within normal limits. Roentgenologic visualization of the gallbladder after oral administration of dye revealed a functioning bladder containing stones. Cholecystectomy was advised, but the patient was not seen again until Feb. 12, 1946.

The interim history was irrelative. At the time of her return the spleen was palpable 1 fingerbreadth below the left costal margin. The results of urinalysis and blood counts were again within normal limits.

Cholecystectomy and splenectomy were performed on February 21. The surgeon reported a thin-walled gallbladder with palpable stones. The cystic and common ducts were normal. The spleen was removed because it contained a definite tumor. The pancreas and the liver were normal. The uterus was slightly enlarged.

The patient made an uneventful recovery. Complaints referable to the left knee, injured in the automobile accident, then were investigated, and treatment was given. She was dismissed from the clinic on March 3, 1946.

Pathologic Data.—The spleen weighed 500 Gm. The greater part of the organ was occupied by the tumor, which was an almost perfect sphere measuring 7 cm. in diameter. On section the tumor was dark and homogeneous in structure (fig. 1). It was completely encapsulated by a narrow rim of fibrous tissue which was continuous with the splenic capsule. The tumor was thus well demarcated from the splenic tissue, which was situated at one pole of the organ.

Microscopic examination of many sections revealed that some areas were completely obscured by a process of infarction. There was, however, persistence of a definite pattern within the tumor (fig. 2). This pattern consisted of small and medium-sized ramifying spaces or channels lined by endothelium. Between the spaces there was a fibrous stroma in which were found free cells. These cells were of medium and large size and contained round, oval or elongated, pale-staining nuclei. Frequently encountered were relatively normal malpighian corpuscles and

sinusoids. The splenic capsule surrounded the tumor. The tissue lying outside the tumor at one pole appeared to be normal spleen. A diagnosis of hamartoma of the spleen was made.

Case 2.—A woman 72 years old registered on Oct. 2, 1923. She complained of a lump in the right breast, which she had first noticed two months previously. The past history revealed nothing of note. The significant findings were as follows: The systolic blood pressure was 128 mm. of mercury and the diastolic 86. The

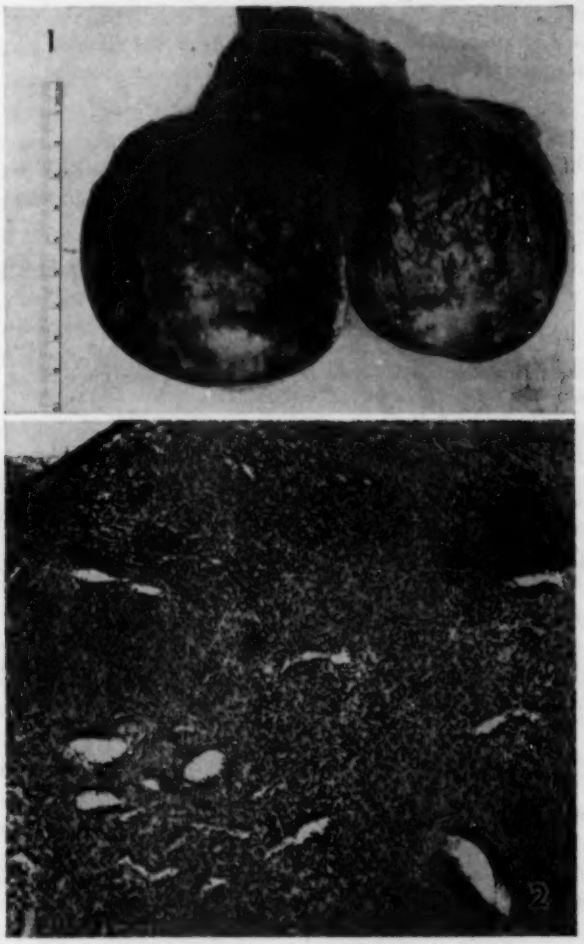

Fig. 1 (case 1).—Hamartoma of the spleen. The tumor was an almost perfect sphere and was surrounded by a thin layer of fibrous tissue before bisection.

Fig. 2 (case 1).—In the upper right corner of the hamartoma is a relatively normal malpighian body. Endothelium-lined spaces of varying size can be seen throughout the remainder of the illustration (hematoxylin and eosin; × 50).

pulse was irregular in force, rate and rhythm; dyspnea occurred on exertion. A fixed hard mass which had caused retraction of skin was palpated in the right breast. Enlarged lymph nodes were present in the right axilla. The left side of the

abdomen was filled by a mobile hard tumor about the size of a grapefruit (10 cm. in diameter). The concentration of hemoglobin and of blood urea and the erythrocyte and leukocyte counts were within normal limits. An electrocardiogram revealed auricular fibrillation with premature ventricular contractions.

A clinical diagnosis of carcinoma of the right breast extending to the axilla, tumor of the spleen or the left kidney, and generalized arteriosclerosis with myocardial degeneration was made. Because the risk of the operation was high, it was decided to treat the lesion of the breast with radium and roentgen rays and to explore the abdominal cavity after induction of local anesthesia.

Splenectomy was performed on October 10. The surgeon encountered a hard tumor of the spleen, involving 90 per cent of that organ. The contents of the abdominal cavity revealed no metastases of the lesion of the breast.

The postoperative course was stormy and was complicated by periods of unconsciousness, decrease of blood pressure and auricular fibrillation of increasing severity. Death occurred November 1, and was attributed to myocardial failure. Permission to make a postmortem examination was not granted.

Pathologic Data.—The spleen weighed 850 Gm. and was roughly spherical in shape, measuring 10 cm. in its greatest diameter (fig. 3). On section the tumor appeared to occupy most of the specimen; it was a dark brown mass with mottled parts of light brown tissue, and it measured 8 cm. in diameter. It was completely surrounded by fibrous tissue which was apparently continuous with the capsule of the spleen. At one pole of the spleen a subcapsular rim of thickened tissue 2 cm. in width, lay outside the tumor and had a homogeneous light brown appearance.

Microscopically the tumor was very similar to that observed in case 1. The spaces, however, tended to be larger (fig. 4), and the malpighian corpuscles were not as numerous. Pigmentation was a notable feature. The subcapsular rim of tissue at one pole of the spleen appeared to be relatively normal spleen. The diagnosis of hamartoma was made.

Case 3.—A woman 41 years old first registered at the clinic on June 17, 1940. For six years she had been conscious of a dull heavy sensation in the left upper quadrant of the abdomen. At the time of her visit she was in the third month of pregnancy, and in the course of a prenatal examination elsewhere a mass had been discovered in the upper left quadrant of the abdomen. The patient had three children living and well. She had had an ovarian cyst removed in 1928.

The findings on examination were consistent with those expected in the third month of pregnancy. In addition there was a firm irregular mass in the upper left quadrant of the abdomen. The borders of this mass could not be definitely traced. Urinalysis and a flocculation test for syphilis gave negative results. The concentration of hemoglobin was 10.2 Gm. per hundred cubic centimeters of blood. Erythrocyte, leukocyte and differential cell counts were within normal limits.

Splenectomy was performed on June 25. The surgeon reported an irregular spleen with a huge hilus and with many large vessels extending throughout the under surface. An enlarged uterus was noted. The pancreas appeared normal, but there was some evidence of mild hepatitis. The patient made an uneventful recovery and was dismissed on July 15.

From correspondence it was learned that in November 1940 a normal delivery occurred at full term.

The patient was admitted for the second time on March 3, 1945. In January attacks of chills and fever had occurred for three weeks. Jaundice, pruritus, anorexia, nausea and a clay color of the stools had developed and persisted. The patient

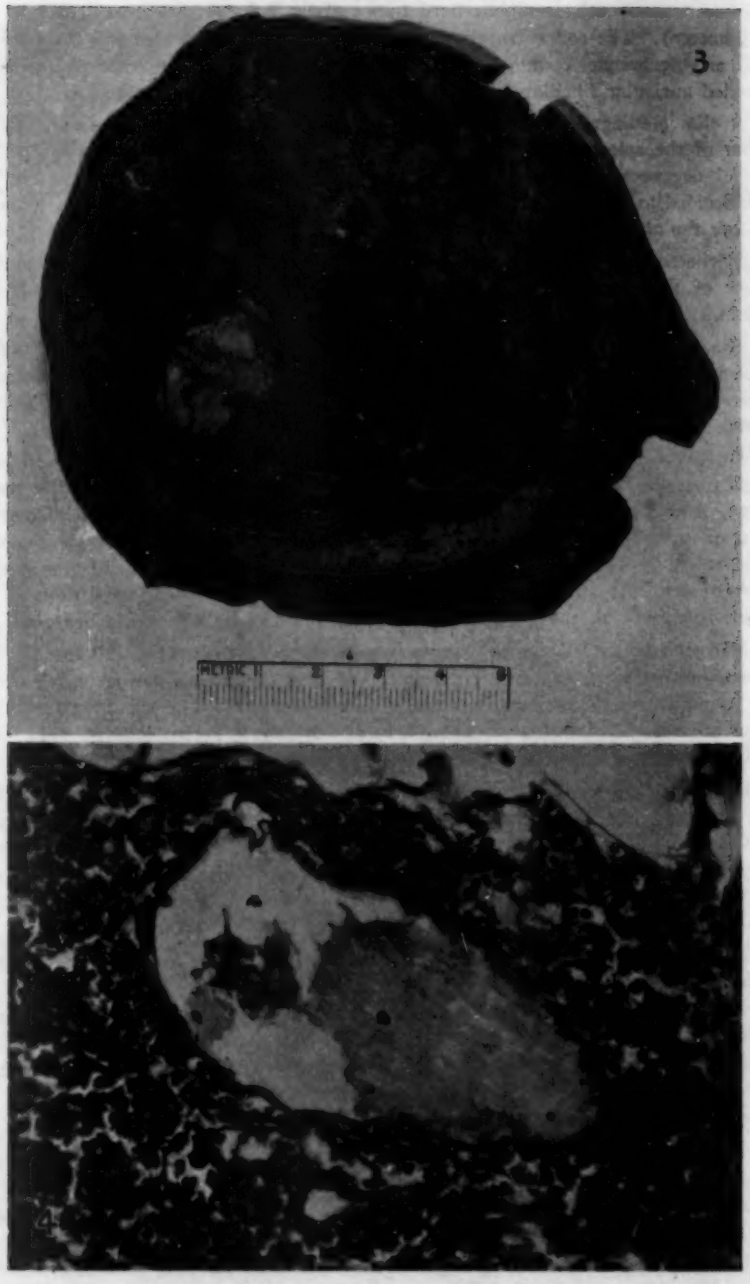

Fig. 3 (case 2).—The spherical hamartoma occupies almost all of the cut surface of the spleen. At the right, the rim of normal splenic tissue can be seen.

Fig. 4 (case 2).—One of the larger spaces which were frequently seen in this hamartoma of the spleen (hematoxylin and eosin; × 390).

had lost 35 pounds (15.9 Kg.) during this period. Just prior to admission an attack of excruciating epigastric pain had occurred and lasted for six to eight hours.

The skin and the scleral coats of the eyes were jaundiced (grade 2, on a grading basis of 1 to 4). The edge of the liver extended about 7 cm. below the right costal margin, and a distended gallbladder could be palpated. Urinalysis gave negative results except for a trace of bile on one examination. The concentration of hemo-

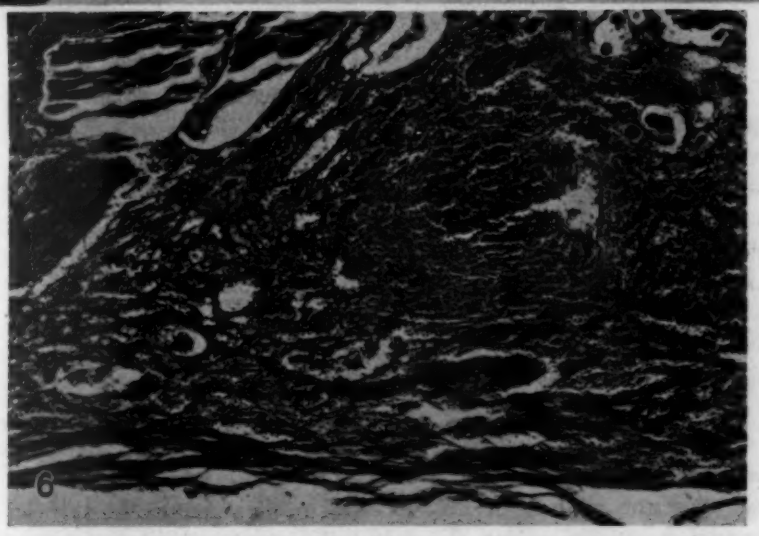

Fig. 5 (case 3).—Hamartoma of the spleen. The entire cut surface of the spleen is involved by lobulated tumor masses.

Fig. 6 (case 3).—Hamartoma of the spleen. Just to the right and above the center is a malpighian body. Spaces of varying size occur throughout the illustration, and the splenic capsule is visible at the bottom (hematoxylin and eosin; × 50).

globin was 8.2 Gm. per hundred cubic centimeters of blood. Erythrocytes numbered 2,800,000 per cubic millimeter of blood and leukocytes 14,700; the differential count was within normal limits. The value for serum bilirubin was 12.9 mg. per hundred

cubic centimeters when the van den Bergh reaction was direct and 2.0 mg. when the reaction was indirect. The prothrombin time was forty seconds. Bile was found in the duodenal drainage.

A transfusion of 500 cc. of whole blood was given on each of two occasions.

On March 14 cholecystogastrostomy was performed. The surgeon reported a greatly distended gallbladder and a dilated common duct. The pancreas was firm and hard throughout its entire extent. No stones were palpated in the gallbladder or the ducts. An uneventful convalescence followed. The serum bilirubin was 2 mg. per hundred cubic centimeters (direct reaction) at the time of the patient's dismissal, April 4.

It was learned from correspondence that death occurred on December 1.

Pathologic Data.—The spleen weighed 1,100 Gm. The surface of the organ presented closely spaced elevated nodules measuring 1 mm. to 2 cm. in diameter. The cut surface was lobulated and contained fairly well outlined dark areas which measured 1.5 to 2 cm. in diameter (fig. 5). These were separated by thin trabeculae.

Microscopic examination showed the pattern of endothelium-lined spaces which was seen in cases 1 and 2. The spaces, however, were much larger than in either of the aforementioned cases (fig. 6). Moreover, many were filled with well preserved erythrocytes, which suggested that the tumor was taking part in the splenic circulation. Malpighian corpuscles were infrequent. Apparently the whole spleen was involved by the tumor, as there was no normal splenic parenchyma. The diagnosis was hamartoma of the spleen.

COMMENT

The 3 cases had certain interesting features in common. In cases 1 and 2 the tumor was almost a perfect sphere, clearly demarcated from the remaining splenic tissue and occupying most of the spleen. In case 3 it occupied the entire spleen as far as could be determined. In all 3 cases the tumor showed normal components of the spleen, the splenic capsule, the malpighian corpuscles and the sinusoids, in varying amounts. In each specimen the preponderance of channels lined by endothelium suggested the abnormal mixture of normal tissue elements which Albrecht designated "hamartoma." Moreover, the microscopic appearance of the tumor in these cases corresponded with the microscopic appearance of hamartoma as described by von Falkowski.

The classification of these cases as instances of hamartoma rather than of hemangioma may be a departure from established custom. In discussing the genesis of hemangioma in their report of 6 cases of hemangioma of the spleen Pines and Rabinovitch [11] cited the theories of origin of hemangioma from the literature. These fall into two main groups. One group suggests mechanical forces, such as hypertension, with subsequent dilation of preexisting vessels, and the other comprises several closely related theories suggesting disturbances of the mesenchymal anlage of the vascular elements. The theories included in the latter group are strikingly similar to the concepts of Albrecht and von

11. Pines, B., and Rabinovitch, J.: Arch. Path. **33**:487, 1942.

Falkowski concerning the origin of hamartoma. It is almost inconceivable that a mechanical force dilating preexisting vessels could account for the large circumscribed tumor containing normal splenic elements encountered in our cases. It seems, therefore, that tumors of this type should be classified as hamartoma and that at least 1 of the 6 tumors in the series of Pines and Rabinovitch could be more accurately included in this group. That tumor was of considerable size and was well demarcated from the remainder of the spleen. It contained the same spaces and lining cells as our specimens, and it also contained malpighian corpuscles.

SUMMARY

A review of the literature indicates that hamartoma of the spleen has been recognized infrequently. A search of the files of the Mayo Clinic revealed 3 cases in which splenectomy was performed for splenic hamartoma. These cases are reported in detail. It is suggested that hamartoma is a specific benign tumor and that some cases of hemangioma of the spleen previously reported could be classified more accurately as cases of hamartoma.

DIFFUSE PLASMA CELL MYELOSIS

Report of a Case in Which It Simulated Aplastic Anemia on Postmortem Examination

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MULTIPLE myeloma has been classified by Ulrich [1] as follows: (1) solitary benign tumors, either osseous or extraosseous; (2) single or multiple malignant tumors of bones, with or without extraosseous growth; (3) diffuse myelomatosis of the marrow, with or without infiltration of other organs, and (4) "plasmacytic leukemia." Apitz' [2] classification is essentially the same. Of these groups, the tumors comprising the first two are relatively common, while those of the last are not rare. Diffuse myelomatosis is quite uncommon, and the patients whose cases have been reported have almost uniformly had either gross evidence of replacement of marrow or "myeloma cells" in the peripheral blood.[3]

While the occurrence of a true diffuse plasma cell myelomatosis, or myelosis, has been mentioned by numerous writers, actual reports of cases are rare. Freund [4] reported the case of a 72 year old woman in whom a diffuse plasma cell involvement of the marrow was not recognized grossly because there was no tumor formation in the bones. The many extramedullary amyloid tumors found in this patient led to the microscopic examination of the marrow and the discovery of a diffuse plasma cell myelomatosis. The author emphasized that the diffuse myeloma is distinguished from the usual types by the fact that it does not produce tumors, and he mentioned that, because of this, similar cases had probably gone unrecognized.

Apitz,[2] in his classification of multiple myeloma, described the marrow seen in cases of the diffuse type as gray and aplastic in appearance

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*Hematology Research Foundation Fellow.

This study was aided by a grant from the Wilson Laboratories.

From the Hematology Laboratory, Department of Pathology, and the Hektoen Institute for Medical Research of the Cook County Hospital.

1. Ulrich, H.: Arch. Int. Med. **64**:994, 1939.

2. Apitz, K.: Klin. Wchnschr. **19**:1025, 1940.

3. Churg, J., and Gordon, A. J.: Arch. Path. **34**:546, 1942. Prentiss, R. G.: Mil. Surgeon **80**:294, 1937. Ulrich.[1]

4. Freund, E.: Frankfurt. Ztschr. f. Path. **40**:401, 1930.

rather than destroyed. He failed to report any cases of his own but pointed out that the disease may easily be missed at necropsy because there is no localized lesion. He expressed the belief that preceding the introduction of marrow aspiration, cases of this type were undoubtedly overlooked.

A Cabot case [5] appears to have been similar to our own. The patient was a 50 year old man who presented himself with diarrhea, vomiting and weakness of two months' duration. Bence Jones protein was identified in the urine, and there was rather vague roentgen evidence of bone destruction in the acetabulum, the skull, the ribs and the vertebrae. The postmortem diagnosis was diffuse multiple myeloma, myeloma of the kidney, bronchopneumonia and cholelithiasis. In the discussion of the case, the condition was described as diffuse myeloma involving all the bones but without any discrete tumor nodules. Interestingly enough, the osseous lesions shown by roentgenograms were not demonstrated at necropsy. No mention is made of the gross appearance of the marrow, the extensiveness of the involvement or the rarity of this type of the disease.

The case reported here was one of diffuse myelomatosis in which there was an almost complete plasma cell replacement of marrow cells without any evidence of tumor formation and without plasma cells in the peripheral blood.

REPORT OF CASE

A 49 year old Negro was admitted to the Cook County Hospital complaining that he had suffered from "indigestion" for one and a half years, constipation for one year, and bloody stools, cramps in the legs, and a 25 pound (11 Kg.) loss of weight in the previous six months. He also complained of occasional weakness from the waist down.

Examination revealed a well nourished Negro man who did not appear acutely ill. He was cooperative and well oriented, although slightly euphoric. The mucous membranes were pale. The blood pressure was 120 systolic and 70 diastolic; the pulse rate, 84; the respiratory rate, 18, and the temperature, 97.8 F. The tongue was coated. The heart was normal. There was decreased tactile fremitus, with dulness to percussion, over the base of the right lung, and a pleural rub was heard in the same area. The abdomen and the genitalia revealed no abnormalities. External hemorrhoids were present, and varicosities of both lower extremities, but no edema.

The impression was one of pleural thickening at the base of the right lung and anemia, with pathologic changes in the gastrointestinal tract, chronic cholecystitis and chronic nephritis to be ruled out.

The blood cell count and the blood film were suggestive of pernicious anemia: hemoglobin content, 20 per cent (3.1 Gm.); red cells, 1,290,000; white cells, 1,700, with a differential count of 80 per cent neutrophils, 19 per cent lymphocytes and 1 per cent monocytes. The platelets were decreased in number. The red cells were macrocytic and slightly hypochromic, with moderate poikilocytosis. Since the clinical appearance of the patient did not corroborate the tentative diagnosis, a puncture aspiration of the sternal marrow was performed. The marrow was very cellular, with plasma cells present almost to the complete exclusion of all other cells except megakaryocytes. A diagnosis of the plasma cell type of multiple myeloma was made.

5. Multiple Myeloma, Diffuse, Cabot Case 21082, New England J. Med. **212**: 353, 1935.

The laboratory work-up yielded the following results: urine—specific gravity 1.008, albumin 3 plus, microscopic data normal, Bence Jones protein absent; the Kahn test of the blood negative; blood nonprotein nitrogen 33 mg. per hundred cubic centimeters; total serum proteins 6.2 Gm. per hundred cubic centimeters.

Gastrointestinal roentgenograms revealed no abnormalities. A roentgenogram of the chest showed a fairly dense irregular opacity in the medial portion of the base of the right lung. The bones seen in these roentgen films were normal.

The patient received 500 cc. of whole blood. Before roentgenograms of the skeletal system could be obtained he had a sudden massive hematemesis and died.

Autopsy.—The sternum, vertebrae, the pelvic bones and all ribs were examined. The red marrow was pale and atrophic, even a little gelatinous. The calvarium showed no changes. The long bones were not examined.

The rugae of the stomach were flattened. Small areas of submucosal hemorrhages, measuring up to 5 mm. in diameter, and many small, shallow erosions of the mucosa, measuring 2 to 3 mm. in diameter, were found.

The spleen weighed 110 Gm. and on cut section was a pale red. The liver weighed 1,330 Gm. and was a pale gray-brown with reddish acinous centers. The heart showed no marked abnormalities. Areas of old caseous tuberculosis were present in the lung.

Microscopic Examination.—Sternum: The marrow (fig. 1) was rather cellular, and only in a few areas was there much fat. The bony structure was not altered. Plasma cells with dense nuclei of varying size were predominant. Owing to the high degree of density the spoke wheel structure was only rarely made out. Some cells contained two and some even three nuclei. The homogeneous cytoplasm also varied in amount but was rarely abundant. The cells appeared to be discrete and most of them were round, even where they were arranged in strands between the fat tissue (fig. 2). A variable number of reticulum cells, and many megakaryocytes were seen. Myeloid and erythroid elements were almost totally absent, but when present they were arranged around the capillaries. Few lymphocytes were seen.

Rib: The marrow was very cellular, with marked preponderance of plasma cells. Large numbers of megakaryocytes, normal in appearance, were also encountered here. In addition, myeloid elements arranged in clusters were noted, as were diffusely distributed erythroid elements. An occasional acute necrotizing miliary tubercle was also noted in the marrow.

Spleen: The follicles were small and without germinal centers. The pulp was rich in blood and contained many proliferating reticulum cells. Rather densely scattered tubercles, with central caseation, surrounded by giant and epithelioid cells, were seen.

Liver: The structure was well preserved. Kupffer cells were mobilized but were usually of winged shape. Their cytoplasm was occasionally large and homogeneously eosinophilic, and contained granular material. The liver cells of the centers of the lobules contained brown pigment. There were irregularly scattered tubercles showing wide central necrosis, relatively few epithelioid or giant cells and an almost complete absence of a lymphocytic ring. In one area the necrosis involved the wall of a small branch of a portal vein and the caseous material had broken through into the lumen of the vein.

Kidney: The structure of the cortex was somewhat obscured by glomerular scars in areas of interstitial fibrosis surrounding the atrophic tubules. A distinct lobulation of the glomeruli as a result of the fusion and thickening of the basement membrane of the glomerular loops was found. The tubules were often dilated and

were filled with thick albuminoid material varying from a pink to a bluish hue. In some areas the lining epithelial cells were partially sloughed or atrophic, while in other areas they showed regeneration with production of giant cells. The tubular lining was rather irregular in some areas, and, interstitially, infiltrative histio-

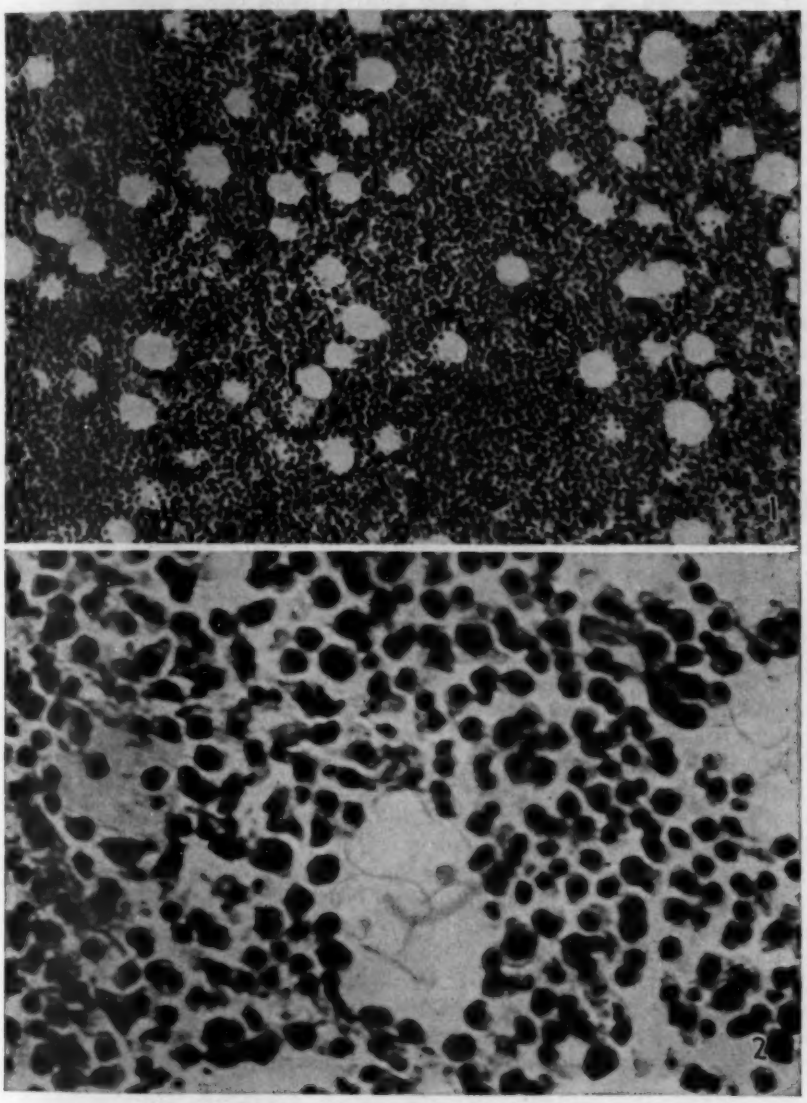

Fig. 1.—Marrow with plasma cells completely replacing all other cells except the megakaryocytes.

Fig. 2.—Marrow showing homogeneous plasma cell replacement.

cytic and leukocytic elements were often seen around lumps of reddish material within the interstitial tissue. Many of the tubules appeared completely obstructed.

In the medulla there was marked widening of the interstitial tissue, which assumed a homogenous reddish hue. Cellular infiltration was seen only in a few areas.

Lung: Several sections were available. One, apparently from the apex, revealed anthracotic thickening of the septums with emphysematous dilatation of the alveoli, many of which were filled with red blood cells. Below the pleura, which was markedly scarred, there was an anthracotic fibrotic area. Another section showed hyalinized tissue with diffuse anthracotic deposits. In this area were ghost remnants of tubercles and fibrosing lung tissue. In a third section a large caseating area was seen under the thickened and hyalinized pleura. In this recently caseated zone the remnants of fibrosing pulmonary tissue as well as of tubercles were well made out. On the periphery, either active caseating granulation tissue with epithelioid and Langhans giant cells or circumscribed smaller or larger, centrally caseating tubercles were seen. Some pulmonary veins contained caseous material in their lumens. The preserved alveoli in the vicinity were filled with desquamated alveolar cells.

Heart: The muscles fibers revealed no abnormalities. The pericardium was thickened and fibrotic and occasionally contained focal cellular infiltration around anthracotic material. The cellular infiltration extended into the superficial layers of the myocardium. Most of the cells were small and round but were not plasma cells.

COMMENT

Aside from the apparent rarity of the condition, this case was of particular interest because of the gross appearance of the marrow. Although the diagnosis had been established ante mortem, the examining pathologist was unable to substantiate it grossly at necropsy. The gray appearance of the marrow, the difficulty with which it was cut, the normal appearance of the trabeculae, the failure to find any evidence of tumor formation, even on diligent search, and the absence of extraosseous involvement, all pointed to the diagnosis of aplastic anemia. The previously obtained marrow specimens, however, disproved this impression, since they showed the megakaryocytes to be intact in a cellular marrow made up almost entirely of plasma cells, whereas had this been aplastic anemia, all the cellular elements should have been markedly depleted and replaced by fat or fibrous tissue. The microscopic observations of the marrow and the changes noted in the kidney confirmed the antemortem diagnosis.

It is only logical to speculate on the role of the pulmonary and miliary tuberculosis. That the findings represented a plasma cell reaction to tuberculosis is inconceivable on the basis of the changes observed in the marrow and the kidney. Contrariwise, it is highly probable that the general myelosis, by virtue of the secondary debility, the loss of weight and the anemia, allowed a latent or a partially healed pulmonary tuberculosis to become activated and to spread. This sequence of events is by no means uncommon in chronic progressive blood dyscrasias.

SUMMARY

A case of diffuse plasma cell involvement of the marrow without any localizing lesions, tumor formation or peripheral plasmacytosis is reported. The difference between this and the confluent type of multiple myeloma is emphasized. Attention is called to the ease with which the condition may be mistaken for aplastic anemia.

General Reviews

SPLENOMEGALY

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THE SPLENOMEGALIES are divisible into acute and chronic forms. Further than this there seems to be no satisfactory method of classification, largely because a number of diseases which are attended by splenomegaly are themselves not yet acceptably classifiable. However, for convenience sake the splenomegalies may be assembled in groups and presented as follows:

Infective and parasitic splenomegalies—acute splenitis (acute splenic tumor), tuberculosis, Boeck's sarcoid, syphilis, kala-azar, visceral leishmaniasis, ponos, malaria, schistosomiasis (Egyptian splenomegaly, bilharziasis).

Splenomegalies of circulatory origin—chronic passive congestion, speckled spleen (Fleckmilz).

Mechanical splenomegaly—polycythemia vera (primary polycythemia, erythremia, Vaquez-Osler disease).

Metabolic splenomegalies—Gaucher's disease, Niemann-Pick disease, Letterer-Siwe disease, Hand-Schüller-Christian disease, primary xanthomatosis, amyloidosis, including Still's disease (multiple rheumatoid arthritis).

Blood dyscrasias—lymphoid, myeloid and eosinophilic (myeloid) leukemia; congenital hemolytic icterus, idiopathic thrombopenic purpura, pernicious anemia.

Splenomegalies of unknown nature—giant follicular lymphadenopathy, Hodgkin's disease, Briquet's gastrointestinal pseudoleukemia, Banti's disease, agnogenic myeloid metaplasia.

Neoplasmic and cystic splenomegalies—noncancerous tumors, spindle cell sarcoma, lymphosarcoma, Kaposi's sarcoma, reticuloendotheliomatosis, metastatic tumors, traumatic cysts, parasitic cysts.

INFECTIVE AND PARASITIC SPENOMEGALIES

Acute Splenitis.—Acute hyperplastic splenitis, or acute splenic tumor, occurs in various infective diseases, including typhoid fever, streptococcic sepsis, pyemia, bubonic plague and anthrax septicemia.

From the Laboratories of Pathology, Bellevue Hospital.

In such circumstances the spleen seldom attains a weight of more than 500 Gm. The substance is soft or even diffluent. Histologically, the changes consist largely of intense hyperemia and diffuse exudation of polymorphonuclear neutrophils.

Tuberculosis.—Tuberculosis of the spleen may be "primary" or secondary. According to Winternitz [1]

> . . . By primary it is not meant that the spleen is the portal of entry of the organism, or even the first tissue of the body in which the disease manifests itself, but that the disease process may localize itself in the spleen and not only bring about various lesions, but act as a focus for dissemination of the bacilli, while the original focus may be healed entirely. It is this type of the disease which we may speak of tentatively as primary tuberculosis of the spleen.

In other words, Winternitz seems to be in agreement with the many other pathologists who maintain that primary tuberculosis of the spleen does not exist. According to Winternitz, so-called primary tuberculosis of the spleen occurs most often between the ages of 20 and 40 but has been encountered in the first year of life and in the eightieth. It is equally distributed between males and females. The disease may first manifest itself in at least three different ways. In one group of cases the onset is characterized by pain or by tumefaction, or by both, in the region of the spleen. This is the commonest method of onset and occurs in about 70 per cent of the cases. In another group of cases the symptoms of onset are those of an acute infective process characterized by, among other things, chills and fever. In a third group the earliest symptoms and the course of the disease are intermediary between the two groups mentioned. In 31 of the 51 cases collected by Winternitz the spleen was weighed. The smallest spleen weighed 150 Gm. and the largest 3,780 Gm. The average weight was 1,350 Gm. Caseous masses were present in 23 of the 51 cases. In 80 per cent of the cases in which the liver was examined tuberculosis was present; the lymph nodes were involved in 57 per cent and the lungs in 40 per cent. In 16 per cent of the cases in which pulmonary tuberculosis was present the lesions were apparently healed, while in 24 per cent the tuberculous process was still active. In many quarters so-called primary tuberculosis of the spleen is regarded as an indication for splenectomy. If, for example, the liver is also involved in the tuberculous process, removal of the splenic seed bed relieves the liver of innumerable fresh infections, and the organ is thus better prepared to take care of the tuberculous foci which are already present in it. In so-called primary tuberculosis of the spleen "death invariably follows if the spleen is not removed. In the cases in which splenectomy was performed, the results are remarkable; 59 per cent recovered" (Winternitz).

1. Winternitz, M. C.: Arch. Int. Med. **9**:680, 1912.

Secondary tuberculosis of the spleen is most often encountered in the form of acute or chronic miliary tuberculosis, rarely as large, solitary tuberculomas. In acute miliary tuberculosis the spleen is hyperemic and riddled by young tubercles. In these circumstances the spleen may attain a weight of 800 or 1,000 Gm.

Boeck's Sarcoid.—Boeck [2] (1899) originally described sarcoid as a disease that is characterized by multiple benign nodular lesions of the skin of unknown cause associated with enlargement of superficial lymph nodes. This conception has since been extended to include similar, if not identical, changes in practically every organ in the body, among them the deep as well as the superficial lymph nodes and the spleen. In some cases the spleen may attain a weight of from 700 to 1,000 Gm. Histologically, the changes in sarcoid bear a noteworthy resemblance to those in tuberculosis. In some cases, the giant cells in the tubercle-like formations of sarcoid contain deposits of calcific material such as are seldom if ever encountered in the giant cells of tuberculosis. However, giant cells are not always observed on histologic examination of the lesions of sarcoid and when present do not always contain calcific inclusions. Nevertheless, discussion concerning the nature of the disease is focalized almost exclusively on whether sarcoid is a form of tuberculosis. In 1910 Kyrle [3] and Morawetz,[4] independently of each other, and in 1914, Sweitzer,[5] injected emulsified sarcoid tissue into guinea pigs and produced lesions in them which were histologically indistinguishable from those of tuberculosis. The validity of these experiments has since been challenged because no precautions were taken to prevent cage infections. In 1941 Longcope [6] reported that when sarcoid tissue was inoculated into guinea pigs, rabbits and pigeons no evidence was obtained to support the view that the disease is caused by human, bovine or avian tubercle bacilli. Furthermore, in cases of sarcoid it has been shown that plasma proteins are increased, especially globulin. However, Seibert and her co-workers [7] have shown that increase of the globulin fraction also occurs in tuberculosis. Histologically, the changes in the enlarged spleen of the patient with Boeck's sarcoid correspond to those encountered in the skin, the lymph nodes, the lungs, the liver and other organs.

Syphilis.—The spleen is palpably enlarged, occasionally tender, in approximately one third of all patients with an early form of acquired

2. Boeck, C.: J. Cutan. & Genito-Urin. Dis. **17**:543, 1899.
3. Kyrle, J.: Arch. f. Dermat. u. Syph. **100**:375, 1910.
4. Morawetz, G.: Arch. f. Dermat. u. Syph. **102**:109, 1910.
5. Sweitzer, S. E.: J. A. M. A. **63**:991, 1914.
6. Longcope, W. T.: J. A. M. A. **117**:1321, 1941.
7. Seibert, F. B.; Seibert, M. V.; Atno, A. J., and Campbell, H. W.: J. Clin. Investigation **26**:90, 1947.

syphilis (Wile). The enlargement is due presumably to participation of the splenic lymph follicles in the hyperplasia which occurs in the lymph nodes and other lymphoid structures in the eruptive stage of syphilis. Enlargement of the spleen occurs in about 16 per cent of all cases of congenital syphilis, ranking second in incidence only to the splenomegaly of rickets (Carpenter). That this form of splenomegaly may persist is indicated by the fact that it is frequently associated with deferred congenital syphilitic cirrhosis of the liver or with hepar lobatum in young adults.

During a period of thirty years that form of syphilitic cirrhosis of the liver which is known as hepar lobatum or botryoid liver was encountered 102 times among 23,792 necropsies at Bellevue Hospital (Symmers and Spain,[8] 1946). In 47 cases, at necropsy, the spleen was described as "normal in size," "slightly enlarged," "moderately enlarged" or "considerably enlarged." In an additional 55 cases the average weight of the spleen was 538 Gm., or 338 Gm. in excess of the extreme normal limit of 200 Gm. The smallest spleen weighed 24 Gm. and occurred in a 9 year old subject with congenital syphilis. Among adults the smallest spleen weighed 60 Gm. and the largest 3,410 Gm. In 18 of the 102 cases of hepar lobatum the spleen showed the changes incident to chronic interstitial syphilitic splenitis, and in 3 cases deposits of amyloid were present.

There is a splenomegaly which occurs in persons with a late form of acquired syphilis, perhaps exclusively, that is attended by small ochre-colored bodies consisting microscopically of thickened and hyalinized central arteries with mineralization of their fibromuscular and elastic coats, together with mineral-free hyaline deposits in and around neighboring venules, as well as periarterial hemorrhagic extravasations. It appears that up to 1919 only 9 cases of this description had been recorded in the literature of medicine—1 each by Marini[9] (1902), Simonds[10] (1908) and Sprunt[11] (1911) and 6 by Symmers, Gettler and Johnson[12] (1919); 2 additional cases have since been recorded in the necropsy reports of Bellevue Hospital and 1 case at Goldwater Memorial Hospital; all of the subjects had syphilitic cirrhosis of the liver or syphilis of the liver and other organs, including fibrosis of the testicles and like residua. The spleens were greatly enlarged, weighing 1,000, 1,280, 775, 1,900, 3,410, 1,275, 1,050, 1,040, 1,150, 1,020 and 1,030 Gm., an average of 1,357 Gm. or 1,157 Gm. above the high normal

8. Symmers, D., and Spain, D. M.: Arch. Path. **42**:64, 1946.

9. Marini, G.: Arch. per le sc. med. **26**:105, 1902.

10. Simonds, J. P.: J. Infect. Dis. **5**:23, 1908.

11. Sprunt, T. P.: J. Exper. Med. **14**:59, 1911.

12. Symmers, D.; Gettler, A. O., and Johnson, W. M.: Surg., Gynec. & Obst. **28**:58, 1919.

weight of the adult spleen. In 1 case the weight was not recorded, but the spleen was described as "three times larger than normal." Of the 6 patients with this condition observed by Symmers, Gettler and Johnson at Bellevue Hospital, 5 were males and 1 a female. The ages were 22, 32, 38, 50, 45 and 29 years. The liver of one subject revealed the naked eye changes of hepar lobatum. The second case was that of a patient with a strongly positive Wassermann reaction associated with generalized superficial lymphadenopathy and bilateral symmetric rupia of the region of the external malleoli, which appeared at intervals for fifteen years, healing slowly only to recur. In a third patient the condition was associated with hepar lobatum and left-sided chronic interstitial orchitis with extensive fibrous replacement. A fourth patient had a syphilitic scar in the liver, irregularly distributed patches of testicular fibrosis and syphilitic changes in the lymph nodes. A fifth had a positive Wassermann reaction and the characteristic microscopic changes of diffuse syphilitic splenitis. The sixth was a man with hepar lobatum and a solitary gumma of the liver.

In this form of splenomegaly it appears that hemorrhage is followed by disintegration of hemoglobin, with the iron compounds being deposited in the hyalinized arterial coats, and that the circulation is impaired in such a manner as to permit escape of serum, with the calcium being precipitated in fibers that have been prepared for its reception by previous ironization. Also, salts of silica and phophorus are present in increased quantities, the former accounting, apparently, for the ochre color of the deposits in the substance of the spleen. This variety of splenomegaly, as observed at Bellevue Hospital, was encountered in syphilitic subjects only. Selective mineralization of the small arteries, while differing from the changes commonly found in syphilitic vessels, appears nevertheless to represent the deposition of salts in tissues that have been injured by syphilis. Whether identical changes occur in vessels in association with diseases other than syphilis remains to be seen.

Splenomegaly due to solitary or multiple gumma occurs but is extremely rare. In a case of this description investigated by necropsy at Bellevue Hospital, the lower pole of the spleen was the seat of a gumma which measured 8 cm. in diameter. This occurred in a prostitute who died of intrapericardial hemorrhage following rupture of a gumma at the apex of the left ventricle.

It appears that splenectomy is sometimes advisable in syphilitic splenomegaly. For example, at Bellevue Hospital a woman aged 29 presented the signs of severe anemia which, she said, had been present since childhood. Rupia were present in the lower parts of the legs. The spleen was greatly enlarged and easily palpable, and caused bulging of the abdominal wall in the left upper quadrant. The red cells

numbered 3,500,000; many normoblasts and a few megaloblasts were present, with polychromatophilia and anisocytosis; the hemoglobin content was 47 per cent. The Wassermann reaction was strongly positive. The rupial lesions were persistently resistant to antisyphilitic treatment, but after splenectomy and continued antisyphilitic treatment these lesions disappeared entirely and there was marked improvement in the anemia.

Kala-Azar.—The term "leishmaniasis" is applied to three diseases known as Indian kala-azar or Dumdum fever, infantile leishmaniasis or ponos, and tropical ulcer or Delhi boil. All of them are caused by slightly different species of protozoan parasites belonging to the genus Leishmania, or Leishman-Donovan bodies. Clinically, the infection is divisible into visceral and so-called dermal forms. Visceral leishmaniasis is characterized among other changes by progressive enlargement of the spleen and often by hepatomegaly. The term "dermal leishmaniasis" implies that the disease is limited to changes in the skin, but adjacent mucous membranes may be involved as in the naso-oral South American form of tropical ulcer.

Visceral Leishmaniasis.—The general appearance of the patient with well established visceral leishmaniasis is not unlike that of the patient with cirrhosis of the liver, including the anxious or concerned expression of the so-called hepatic facies. In visceral leishmaniasis there is progressive loss of weight, the arms, the bones of the face and the ribs being especially prominent. The superficial veins in the anterior abdominal wall are often distended and the abdomen is protuberant because of enlargement of the spleen or of the spleen and the liver and, late in the disease, because of ascites. The skin may assume a blackish hue, "kala-azar" in Hindustani meaning "black poison." The size of the spleen may progress to the extent of almost complete occupancy of the abdominal cavity, and the liver is usually enlarged. At first the spleen is soft or doughy; as the disease progresses the organ becomes firm. In the late stage, anemia is marked, and nucleated red cells may be found in the peripheral blood; in the late stage leukopenia is the rule. The temperature is extremely erratic. In many cases, a "double rise" occurs in twenty-four hours, and the fever is quinine resistant. A patient observed at Bellevue Hospital, a native of India, presented temperature fluctuations simulating those of sepsis. It is obviously important in cases of kala-azar to exclude temperature changes due to concomitant malaria.

The weight of the enlarged spleen varies from 300 to 1,500 Gm. and is said sometimes to reach as high as 4,500 Gm. Histologically, enlargement of the spleen is due partly to congestion with or without areas of infarction and partly to vast numbers of fixed or wandering cells that

are richly parasitized by Leishman-Donovan bodies. According to Meleney [13] (1925), the cells are clasmatocytes which form "tissue masses" often before parasitization of individual cells occurs. In addition, many parasites lie free in the tissues (Stitt [14]).

Ponos.—The disease which the Greeks sometimes call ponos is limited to the period of infancy or childhood and is almost always fatal. It is characterized by fever, painful enlargement of the spleen, cachexia and hemorrhages. According to the earlier descriptions, ponos is restricted to and endemic in the two small islands of Spezzia and Hydra off the coast of Greece. At one time it was thought that the disease was transmitted by drinking rain water. Stitt [14] (1942) quotes Gabbi as having shown in recent years that ponos is a form of Mediterranean leishmaniasis or infantile kala-azar. As a rule, the onset is gradual and is attended by fever (the temperature sometimes reaching 104 F. by nightfall), by pallor and rarely by jaundice. Within a few weeks the spleen becomes palpably enlarged and increases rapidly in size, eventually occupying a large part of the abdominal cavity. The organ is tender and so often painful that the designation πονος, meaning pain, was bestowed by the Greeks as fitting the disease. Gastrointestinal disturbances are frequent; hemorrhages occur, usually from the gums or nose, occasionally in the form of petechiae or ecchymotic splotches or as subperiosteal extravasations, and death ensues in from eight to eighteen months (Davidson,[15] 1912).

Malaria.—The malarial spleen varies in size, color and consistency, depending on the length and the severity of infection. In an early stage of infection the spleen is slightly or moderately enlarged because of intense hyperemia, and the pulp may be diffluent, the lymph follicles sometimes standing out as grayish bodies against a background color of blue or red; at other times the follicles are submerged in the swollen and congested pulp. In older and more severe infection the spleen is enlarged, often to an enormous extent, attaining a weight of 1,500 Gm. or more. It is firm in consistency, and the color is a characteristic grayish or slate blue or even greenish or greenish black, owing to the accumulation of pigment-bearing plasmodia or to pigment lying free among the tissue cells or both. Histologically, the spleen at this stage of enlargement shows variable numbers of red cells containing parasites and pigment. The endothelial cells lining both capillary blood vessels and lymph sinuses are similarly parasitized and pigmented, and great

13. Meleney, H. E.: Am. J. Path. **1**:147, 1925.

14. Strong, R. P.: Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases, ed. 6, Philadelphia, The Blakiston Company, 1942, p. 229.

15. Davidson, A., in Allbutt, C., and Rolleston, H. D.: A System of Medicine, London, Macmillan & Co., Ltd., 1912, vol. 2, pt. 2, p. 606.

numbers of wandering macrophages are packed with pigment. In some cases, the arterioles and capillary blood vessels are thrombosed, and multiple hemorrhagic extravasations or focal necroses ensue. This form of splenic enlargement is colloquially known as ague cake. Rupture of the ague cake may occur spontaneously or as a result of slight trauma, and fatal hemorrhage has been known to follow aspiration of the spleen for diagnosis (Osler; Thayer).

Schistosomiasis (Egyptian Splenomegaly or Bilharziasis).—According to Day [16] (1933), enlargement of the spleen occurs as a "temporary toxic reaction in the early stages of all schistosome infestations." However, when cirrhosis of the liver supervenes in Schistosoma mansoni infection, splenomegaly becomes progressive. Day quotes O'Farrell to the effect that there are considerable differences in the degree of enlargement depending on the extent to which the splenic vein is involved. When the lumen of the vein is occupied by worms or its walls and the surrounding tissues contain ova, "a contracting fibrotic periphlebitis is set up which is capable of causing great splenomegaly," and even thrombosis of the vein may occur. In either event the pulp is deeply congested and eventually becomes overgrown by connective tissue, so that in advanced infection, even in children, the fibrotic spleen may weigh 5,000 Gm. or more. This form of splenomegaly is common in Egypt and to a less extent in other parts of Africa. The patients are almost always children or young adults. The disease causes expansion of the upper part of the abdomen with widening of the costal angle, separation of the rectus abdominis muscles above the umbilicus and upward displacement of the heart. It is often attended by marked secondary anemia, and death occurs with the symptoms and signs of cirrhosis of the liver, including ascites.

SPLENOMEGALIES OF CIRCULATORY ORIGIN

Circulatory disturbances sometimes give rise to a moderate degree of splenomegaly, as in the diseases due to chronic passive congestion of heart, lung and liver. Seventeen nonsyphilitic subjects with Laennec's atrophic cirrhosis of the liver were examined postmortem at Bellevue Hospital; the average weight of the congested spleens exceeded the extreme normal by only 112 Gm.

Speckled Spleen.—In 1921 Feitis,[17] under the caption of *Fleckmilz,* described 2 cases of multiple necroses in which the spleen was speckled with small areas of infarction, which were rounded or irregularly shaped, isolated or intercommunicating. In 1938, Schmeisser and Harris [18]

16. Day, H. B.: J. Trop. Med. & Hyg. **36**:17, 1933.
17. Feitis, H.: Beitr. z. path. Anat. u. z. allg. Path. **68**:297, 1921.
18. Schmeisser, H. C., and Harris, L. C., Jr.: Am. J. Path. **14**:821, 1938.

added 2 similar cases and collected 27 others. The ages of the patients varied from 11 to 63 years, and the condition was equally distributed between males and females. In 15 cases the weight of the spleen varied from 40 to 918 Gm., the average weight being 321 Gm. Of 7 spleens which were not weighed but of which the measurements are given, 5 were over 10 cm. in length, the average length being 12.3 cm., or about 2 cm. above the average length of adult spleens. The condition was associated with chronic nephritis in 21 cases, and in 8 cases, with sepsis, pernicious anemia and pneumonia.

MECHANICAL SPLENOMEGALY

Polycythemia Vera ("Primary" Polycythemia, Vaquez-Osler Disease).—The designation "primary" polycythemia or polycythemia vera is used to distinguish a disease entity from secondary or spurious polycythemia such as occurs in congestive heart failure or in association with sclerosis of the pulmonary artery in Ayerza's syndrome. In the latter forms of polycythemia the increased red cell count and hemoglobin content, splenomegaly, if present, and cyanosis seldom approach the degree to which these changes occur in polycythemia vera with almost monotonous regularity.

Polycythemia vera is characterized by persistent increase in the number of red cells in the circulating blood, by splenomegaly and, as a rule, by cyanosis. The observation of Vaquez, confirmed by Weber and others, that the bone marrow is the seat of increased erythrogenesis seems to afford the most plausible explanation of the process as a whole. In most cases of polycythemia vera the augmented activity of the erythroblastic tissues of the marrow is so well equilibrated that, for the greater part, only mature red cells are thrown into the circulation; a few normoblasts and a casual megaloblast are encountered on occasions, although fairly large showers of megaloblasts have been observed. These facts suggest that the cause of the disease is to be found in the marrow, the reaction of whose hematogenic tissues, more especially the erythroblastic centers, is of much the same character as that of the lymph nodes in lymphoid leukemia and of the marrow in myeloid leukemia; in other words, that polycythemia vera is the mature red cell equivalent of the leukemias, the preponderance of circulating erythrocytes being paralleled by the lymphocytosis of lymphoid leukemia and by the myelocytosis of myeloid leukemia (Symmers and Appelbaum,[19] 1919). In both varieties of leukemia the spleen is practically always enlarged. In myeloid leukemia enlargement is due in great measure to the infiltration of myelocytes discharged from the hyperplastic bone marrow. In lymphoid leukemia the splenomegaly, while caused largely by infiltration of cells

19. Symmers, D., and Appelbaum, E.: Proc. New York Path. Soc. **20**:10, 1919.

derived from the enlarged lymph nodes, is contributed to by proliferation of the lymph follicles normally resident in the spleen itself. In polycythemia vera enlargement of the spleen is occasioned solely by erythrocytic infiltration, the organ acting as a reservoir.

In the greater number of cases of polycythemia vera enlargement of the spleen is present, the organ sometimes attaining a weight of 2,000 Gm. or more, often reaching into the pelvis. Microscopically, this variety of splenic enlargement is marked by congestion of an extreme degree, and the process is apparently best interpreted as an effort on the part of the spleen to withdraw and destroy superfluous red cells and thus mechanically to relieve the vascular embarrassment occasioned by increase in the total volume of cells. The cyanosis may equally well be explained on mechanical grounds, the overproduction of red cells by the bone marrow continued without appreciable interruption, eventuating in capillary engorgement throughout the body, especially in those parts where vasomotor control is least stable, including the vessels of the face, of the tongue and pharynx, and of the extremities, and later throughout the skin. Whether, as has been suggested, the mechanical effects of increased erythrogenesis are reenforced by diminished destructibility of red cells is problematic, both Almoth Wright and Saundby having been unable to determine any change in the hemolytic index. Since there are authentic cases of polycythemia vera with splenomegaly but without cyanosis, it would appear that up to a certain point the spleen is mechanically capable of taking care of the excess of red blood cells but that beyond this point its receptive capacities are transcended and the excess of red cells is distributed elsewhere. Polycythemia is then externalized by distention of the blood vessels of the face, the mouth, the throat and the extremities. In polycythemia vera it appears that splenic enlargement is an aid rather than a hindrance. Splenectomy, therefore, is contraindicated, not alone for the reason suggested but also because experience has shown that in those instances in which the patient survives operation no improvement is observed and, moreover, because in these patients splenectomy is attended by a high mortality due, as a rule, to postoperative venous oozing into the abdomen and from the edges of the surgical wound, death occurring within thirty-six or forty-eight hours. Even in those cases of polycythemia vera in which there are clinical reasons for the belief that the spleen is the seat of "localized" tuberculosis, the indications for splenectomy are questionable since in these circumstances, the tubercle bacillus infection is probably secondary, in many cases at least, to the polycythemia and splenomegaly. On the contrary, in cases of "primary" tuberculosis of the spleen, splenectomy is another story, the operation yielding highly favorable results (Winternitz [1]).

METABOLIC DISEASES

Gaucher's Disease.—Gaucher's disease, or cerebrosidosis, is confined practically exclusively to the organs of the reticuloendothelial system. It occurs more frequently in females, has a familial distribution and especially affects members of the Jewish race. Perhaps the most striking feature of the disease is to be found in the enlargement of the spleen, which may attain a weight of 8,000 Gm. In some cases the bone marrow is involved to an even greater extent than the spleen. The disease may be attended by pigmentation of the skin and, in many instances, by bilateral pingueculae, located oftenest on the temporal sides of the corneas. The disease develops insidiously in childhood and frequently persists into adult life, covering a period of twenty years or even longer. Microscopically, the splenic pulp and sometimes the sinuses contain extremely large mononuclear or occasionally multinuclear cells, the cytoplasm of which presents a glazed or hyaline appearance and is often traversed by minute slitlike apertures or elongated vacuoles, which may contain a light yellowish pigment. This is weakly iron positive, but about its chemical nature little else is known. The cells are resistant to the commonly employed fat stains. Similar changes are found in the cells of the lymph nodes, in Kupffer's cells lining the sinusoids of the liver, and in the marrow. At Bellevue Hospital a patient who presented signs of extensive involvement of the marrow sustained a fracture of the pubic bone while walking down stairs.

Niemann-Pick Disease.—Niemann-Pick disease, sphingomyelinosis or lipoid histiocytosis, is not confined to the reticuloendothelial system but may also involve the adrenal glands, the kidneys, lungs, the pancreas, the large arteries, the adventitial cells of loose connective tissue and the brain. In the last situation the cells resemble those of Tay-Sachs disease (amaurotic familial idiocy, infantile type). The disease occurs only in infancy and childhood, practically always in the Jewish race, is familial and terminates fatally within two years. The skin is often yellowish brown, emaciation progresses and idiocy may develop. The spleen is greatly enlarged but seldom if ever to the same extent as in Gaucher's disease. Histologically, the spleen shows the presence of many large cells, which usually have a single nucleus and foamy cytoplasm. The cells are macrophages and contain rounded vacuoles filled with lipoid that stains with sudan dyes and nile blue sulfate and has been identified as sphingomyelin. In the lymph nodes, the liver, the marrow and other organs the cells present changes almost identical with those in the spleen.

Letterer-Siwe Disease.—Letterer-Siwe disease, also known as aleukemic reticulosis, is a rapidly fatal disorder which is neither hereditary nor familial and occurs exclusively in infants. It is attended by marked splenomegaly, the organ sometimes extending downward to the level of

the iliac crest. The liver is "moderately or considerably" enlarged and there is generalized enlargement of the lymph nodes. A tendency toward hemorrhage, manifested especially in the form of petechiae or purpuric splotches, seems to be a constant feature. Histologically, the changes in the various organs, including the bones, the lungs, the skin and the thymus in addition to the spleen, the liver and the lymph nodes, are marked by excessive production of non-lipoid-storing macrophages. Up to 1936 Abt and Denenholz [20] were able to assemble a total of 9 cases, including 1 of their own.

Hand-Schüller-Christian Syndrome.—That form of xanthomatosis which is known as the Hand-Schüller-Christian syndrome occurs oftenest but not exclusively in children, is not a familial disease and is attended by diffuse or nodular collections of lipid-bearing foam cells in the membranous bones of the skull, in the skin, the liver, the lymph nodes, the spleen and elsewhere. Clinically, the disease is often attended by diabetes insipidus following deposition of lipoid substances in the region of the hypothalamus and by exophthalmos, which may be unilateral or bilateral and is due to a similar deposition occurring in the retrobulbar connective tissues. In a case at Bellevue Hospital, the eyeball was almost completely extruded from its socket. The spleen, as a rule, is only slightly or moderately enlarged. In advanced cases the histiocytes containing lipoid substances are replaced by granulation tissue and the lesions may even become fibrotic.

Primary Xanthomatosis of the Spleen.—Dreyfuss and Fishberg,[21] in 1943, called attention to a form of splenomegaly due to overgrowth of reticulum or "foam cells" and the fact that monoaminophospholipids were accumulated in them, especially lecithin and cephalin. The patient was a child aged 6, whose spleen was removed surgically. "The spleen was enlarged to about three times its normal size" and weighed 190 Gm. Chemically, the spleen was entirely different from that of Gaucher's disease and Niemann-Pick disease. The lesion is apparently a unique variety of lipoid histiocytosis.

Amyloidosis.—In the spleen amyloid deposits usually appear first in the walls of the central arterioles of the lymph follicles, later extending through the pulp, their course corresponding to the distribution of the blood sinuses. The spleen in amyloid disease seldom exceeds 500 Gm. in weight.

Still [14] described a disease of children which is characterized by polyarthritis, generalized lymphadenopathy and splenomegaly. In 9 of 12 cases observed clinically, the edge of the spleen was palpable 2 finger-

20. Abt, A. F., and Denenholz, E. J.: Am. J. Dis. Child. **51**:499, 1936.

21. Dreyfuss, M. L., and Fishberg, E. J.: Am. J. M. Sc. **205**:458, 1943.

breadths below the costal slope. In a series of 12 cases investigated at necropsy, slight or moderate splenomegaly was present in 5, and in all of them amyloid deposits accounted for the enlargement. In other cases of Still's disease investigated at necropsy, amyloidosis of the spleen could not be excluded because the spleens were not examined microscopically. In 1 of 2 cases of Still's disease in which the spleen was the seat of amyloid disease the patient was a boy aged 7; the organ weighed 360 Gm., or 273 Gm. above the normal (Portis,[22] 1938).

BLOOD DYSCRASIAS

Chronic Lymphoid Leukemia.—Current texts of medicine and pathology are apt to lead one to believe that enlargement of the superficial lymph nodes is a frequent, if not a constant and the preponderant, anatomic feature of chronic lymphoid leukemia. Apparently this is not true. Of 36 cases of chronic lymphoid leukemia encountered among 22,537 necropsies at Bellevue Hospital, the superficial nodes were not enlarged in 4 (11 per cent) and were enlarged only slightly in 9 (25 per cent), moderately in 18 (50 per cent) and massively in 5 (14 per cent). The thoracic nodes were not enlarged at all in 18 cases (50 per cent) and were slightly enlarged in 3 (8.3 per cent) and massively enlarged in 15 (41.7 per cent). The abdominal lymph nodes alone were involved in 25 instances, or in 69 per cent, while the abdominal and thoracic nodes together were enlarged in 17 cases, or in 47 per cent.

The weight of the spleen was recorded in 32 of the 36 cases. The lowest weight was 250 Gm.; the spleen was that of a 65 year old woman with great enlargement of the thoracic and abdominal nodes and innumerable focal lymphomas in a liver weighing 1,880 Gm., together with generalized superficial lymphadenopathy. The largest spleen weighed 2,500 Gm. and extended downward to the brim of the pelvis and inward to the midline. It was found in a man aged 32 and was accompanied by "about 100" slightly enlarged retroperitoneal lymph nodes. The average weight of the 32 spleens was 887 Gm. One of the 4 spleens for which weights were not recorded was described as "slightly enlarged." The remaining 3 measured 18, 21 and 22 cm. in length. The spleen was preponderantly or primarily enlarged in 10 cases. In all these cases except the 3 just noted, there were various combinations of enlargements of superficial, thoracic and abdominal nodes. However, the aggregate of these enlargements did not approach the estimated sum total of lymphoid hyperplasias of the spleen.

According to necropsy experience at Bellevue Hospital, in one group of cases chronic lymphoid leukemia shows itself in the form of preponderant or, if one chooses, "primary" enlargement of the abdominal lymph nodes, followed by enlargement of the spleen, and in a second group it

22. Portis, R. B.: Am. J. Dis. Child. **55**:1000, 1938.

produces preponderant or "primary" enlargement of the spleen, followed by enlargement of the lymph nodes of the abdomen alone or of those of the abdomen and the chest together (Symmers,[23b] 1944).

Chronic Myeloid Leukemia.—In chronic myeloid leukemia the spleen may attain a weight of from 2,000 to 10,000 Gm. The capsule and the trabeculae, as a rule, are thickened, and the consistence of the organ is increased. The pulp is richly infiltrated by cells derived from the hyperplastic bone marrow, including myeloblasts, eosinophilic and neutrophilic myelocytes and a sprinkling of megakaryocytes.

Eosinophilic (Myeloid) Leukemia.—Stillman[24] (1912) described a variety of myeloid leukemia in which the predominant cell is a slightly modified polymorphonuclear eosinophilic leukocyte instead of the customary neutrophilic or eosinophilic myelocyte. The patient was a man 27 years of age, in whom the right posterior cervical and the right epitrochlear lymph nodes and the inguinal lymph nodes of both sides were enlarged, together with the liver and the spleen. The number of leukocytes varied from 118,000 to 165,000, and the percentage of eosinophilic ones from 85.8 to 91. The patient remained under observation for only a short time and finally disappeared. The histologic changes occurring in the lymph nodes are not recorded because excision could not be done. However, Giffin[25] (1919) recorded a similar case in greater detail under the heading of "Persistent Eosinophilia with Hyperleucocytosis and Splenomegaly." Clinically, there was slight enlargement of the superficial lymph nodes. A lymph node removed from the axilla showed that the "germinal centers were intact" and that eosinophils "were quite numerous and a few scattered eosinophilic myelocytes were to be found." The spleen, which had been removed at operation, measured 30 by 25 cm. and weighed 2,110 Gm. In it eosinophils were numerous and a few eosinophilic and neutrophilic myelocytes were seen. The leukocytes in the peripheral blood varied between 15,400 and 135,000 and the percentage of eosinophils from 56.7 to 90.7. The patient died, and a necropsy was done. In the anatomic diagnosis "general lymphoid hyperplasia" is included among the postmortem findings, although the necropsy report does not mention the occurrence of enlarged lymph nodes except to say that a node about 3 cm. in diameter was removed from the region of the head of the pancreas. Microscopic examination of it showed intact "germ centers" together with numerous eosinophils and a few eosinophilic and neutrophilic myelocytes and relatively few lymphocytes. An almost identical case

23. Symmers, D.: (*a*) Arch. Int. Med. **4**:218, 1909; (*b*) **74**:163, 1944; (*c*) Arch. Path. **32**:764, 1941; (*d*) **34**:385, 1942.

24. Stillman, R. G.: M. Rec. **81**:594, 1912.

25. Giffin, H. Z.: Am. J. M. Sc. **158**:618, 1919.

of eosinophilic leukemia was described by Shapiro [26] in 1919. The patient was a man 40 years of age who had been ill for five years. The leukocyte count changed gradually from 19,800 cells with 70 per cent eosinophils to 236,000 with 79 per cent eosinophils. At necropsy the spleen weighed 1,268 Gm. and measured 25 by 15 by 4 cm. The liver was likewise enlarged. The inguinal and epitrochlear nodes were palpable "but not greatly enlarged," and no enlarged nodes were found elsewhere. Similar cases have been recorded by McDonald and Shaw [27] (1922), Alexander [28] (1924), Harrison [29] (1930) and Brunlik and Sikl [30] (1930).

Other variants or special forms of myeloid leukemia have been described as basophilic and plasma cell leukemia.

Congenital Hemolytic Icterus.—The term congenital hemolytic icterus is used to denote a familial form of relatively mild chronic jaundice in which, in addition to anemia, the red cells show diminished resistance to hypotonic salt solutions, especially the spherocytes or red cells that have lost their biconcave form. On occasions crises occur in which both jaundice and anemia are profoundly intensified. The spleen is often considerably enlarged. Histologically it is marked by a degree of hyperemia which is seldom if ever surpassed by that observed in the spleen in any other known disease. Splenectomy is almost invariably followed by cure.

Idiopathic Thrombopenic Purpura.—Idiopathic thrombopenic purpura, known also as morbus maculosus or Werlhof's disease, occurs oftenest in females who are in early adult life and is attended by spontaneous bleeding into the skin or from mucous membranes, diminution of the number of thrombocytes, or blood platelets, prolonged bleeding time, normal coagulation time and delayed clot retraction. The spleen is often enlarged. According to Frank and Dameshek and Miller, the fundamental defect leading to thrombopenia is diminished platelet production on the part of the megakaryocytes of the bone marrow, probably due to a form of hypersplenism in which the normal production and discharge of platelets are inhibited. In these circumstances, splenectomy is practically always followed by remarkable alleviation of symptoms if not by cure of the disease. Histologically, in one group of cases, the spleen appears to be normal. In other cases the changes are those of Gaucher's disease, thrombosis of the splenic vein, Boeck's sarcoid, infective splenomegalies including so-called Felty's syndrome

26. Shapiro, L. G.: Proc. New York Path. Soc. **19**:73, 1919.
27. McDonald, S., and Shaw, A. F. B.: Brit. M. J. **2**:966, 1922.
28. Alexander, M. E.: J. Lab. & Clin. Med. **9**:803, 1924.
29. Harrison, F. F.: Am. J. M. Sc. **179**:208, 1930.
30. Brunlik, J., and Sikl, H.: Folia hemat. **43**:1, 1930.

of splenomegaly, leukopenia and rheumatoid arthritis. In a case recorded by Mandelbaum, Berger and Lederer, Gaucher's disease, in a man of 41, was associated with hemolytic anemia and thrombopenia. The spleen, which was removed surgically, weighed 6,822 Gm. Splenectomy was followed by improvement, including remission of anemia and disappearance of pingueculae and of patchlike bronzing of the skin. Dameshek "reports a case of Gaucher's disease and marked pancytopenia in which splenectomy was performed because of a well defined hemorrhagic tendency and because the spleen was so large that it interfered with the child's locomotion. Following splenectomy there was a dramatic response in all the blood elements, which has been sustained for seven years" (Doan and Wright,[31] 1946).

Pernicious Anemia.—In 20 cases of pernicious anemia encountered among approximately 4,800 necropsies at Bellevue Hospital, the spleen was enlarged in 7, or in 35 per cent. The smallest spleen weighed 260 Gm. and the largest 860 Gm. The average weight was 543 Gm. or 343 Gm. in excess of the high normal weight of the adult spleen. In these circumstances enlargement is apparently due in great measure to the accumulation of degenerate red cells, some of which lie free in the pulp, while others are phagocytosed. In the same series of necropsies the hemolymph nodes, which some regard as an accessory system of diminutive spleens, were enlarged six times or in 30 per cent because of the same mechanism of accumulation and autolytic or phagocytic destruction of red cells.

SPLENOMEGALIES OF UNKNOWN NATURE

Giant Follicular Lymphadenopathy.—The comparatively newly described disease that is variously known as giant follicular lymphadenopathy, giant follicle hyperplasia of lymph nodes and spleen, giant follicular lymphoblastoma, giant follicular lymphoma, etc., is characterized anatomically by generalized enlargement of the lymph nodes and, in a considerable percentage of all cases, by splenomegaly. Histologically, the changes in both localities consist of numerical and dimensional hyperplasia of lymph follicles. The question whether there is a form of giant follicular lymphadenopathy which arises in the spleen cannot be put at rest until necropsy reveals an enlarged spleen in which the lymph follicles show numerical and dimensional hyperplasia unattended by extensive enlargement of lymph nodes in other parts of the body likewise due to follicular hyperplasia. A case of possible primary giant follicular lymphadenopathy of the spleen was that of a woman aged 48 who was admitted to Bellevue Hospital Nov. 24, 1937 and again May 3, 1938. On both occasions she complained of tarry stools, which had been present "off and on" for a period of several years, and occasional

31. Doan, C. A., and Wright, C. S.: Blood **1**:10, 1946.

vomiting of large quantities of bright red blood. The spleen was firm and enlarged, extending about 10 cm. below the costal slope on the left side. There were no detectable signs of enlargement of any of the superficial lymph nodes. Splenectomy was done. At the time of operation no enlarged lymph nodes were seen or felt in the abdomen. The spleen weighed 800 Gm. and measured 15 by 8 by 5 cm. Scattered through it were large numbers of whitish areas corresponding histologically to hyperplastic lymph follicles (Symmers,[28d] 1942). Decker and Little [32] (1935) have published a case in which the spleen weighed 3,500 Gm. and measured 22.5 by 11.3 by 6.5 cm. There was no apparent enlargement of the superficial lymph nodes. On microscopic examination the spleen was found to be riddled by hyperplastic lymph follicles. At operation two enlarged nodes were found in the gastrosplenic omentum, the larger measuring 2 cm. in its longest diameter. Microscopic examination of this node showed changes which were practically identical with those in the spleen. Two years and five months later the patient still showed no detectable signs of lymph node enlargement in any part of the body. De Jong has reported 3 cases in which an enlarged spleen was removed at operation, the enlargement of which was due to hyperplastic lymph follicles. No enlarged lymph nodes were detected in any of the 3 cases.

Hodgkin's Disease.—Clinically, in order of their appearance, the changes in the spleen in Hodgkin's disease are oftenest secondary to those in the lymph nodes, but in occasional instances it appears that the burden is borne by the spleen, almost alone. In 1909 Symmers described the case of a girl 18 years of age who for a period of three years before she was admitted to the hospital knew that there was a mass in the left side of her abdomen. The superficial lymph nodes were not detectably enlarged. At the time of her admission the edge of the spleen was felt just above Poupart's ligament on the left side, whence it could be traced to the right as far as the umbilicus and thence upward. The organ was removed at operation and measured 25 by 18 by 8 cm. On microscopic examination it presented the characteristic histologic aspects of Hodgkin's disease. At the time of operation no enlarged lymph nodes were seen or felt in the abdomen. Death occurred, but necropsy was not permitted. Since then Symmers has encountered at necropsies a series of 6 additional cases in which the spleen was greatly increased in size, weighing on an average 1,136 Gm., or 936 Gm. above the high normal. There were a few associated lymph node enlargements, usually in the abdomen but occasionally in the neck, the size and number of which, however, were insignificant as compared with the extent to which the spleen was enlarged. Examples of preponderant or "primary"

32. Decker, H. R., and Little, H. G.: J. A. M. A. **105**:932, 1935.

splenic Hodgkin's disease, likewise verified at necropsy, have since been recorded by Cornwall and l'Esperance.

It is recognized that there is a phase in the clinical evolution of certain cases of Hodgkin's disease that is marked by enlargement of the spleen before the appearance of recognizable signs of involvement of the lymph nodes of the chest, the neck or the abdomen. In addition to the cases just enumerated there was a woman under observation at Bellevue Hospital whose spleen reached into the pelvis and as far forward as the mamillary line (Symmers,[23b] 1944). She was in the hospital for several weeks with the enlarged spleen as the only clinically detectable sign of disease. Finally, a small lymph node became barely palpable in one of the anterior cervical triangles. The node was removed, and microscopic examination showed the histologic changes of Hodgkin's disease. These facts appear to confirm the opinion that there is a variety of Hodgkin's disease in which the preponderant lesions are in the spleen, while in the lymph nodes, if changes are present at all, they are relatively negligible.

Of 30 cases of Hodgkin's disease investigated by necropsy at Bellevue Hospital, the spleen was apparently secondarily enlarged in 20, or in 66 per cent, and in all of them the organ was nodular (Symmers,[23b] 1944). The average weight was 801 Gm., or 601 Gm. in excess of the high normal weight in health. In all of the 20 cases of splenomegaly characteristic histologic changes were present. In 4 other cases the histologic changes in the spleen were those of Hodgkin's disease, but the organ was normal in size or only slightly enlarged and could not be palpated during life. In 4 other cases the spleen was normal both in size and microscopically. In short, in about one fourth of these Bellevue Hospital cases of Hodgkin's disease the spleen was not palpable during life—a fact which appears to be of clinical significance in that, contrary to common teaching, enlargement of the spleen is not a constant accompaniment of Hodgkin's disease.

Pseudoleukemia.—Shortly after the discovery of lymphoid leukemia independently by Bennett and Virchow, Cohnheim, under the title of pseudoleukemia, described a disease presenting the anatomic characteristics of lymphoid leukemia without corresponding changes in the blood, at least according to the hematologic standards of that day. The patient was a man aged 42 in whom the leukocytes were considerably diminished in numbers. At necropsy the spleen was found to be increased in size, measuring 25 by 17 by 8 cm., and presented innumerable hyperplastic lymph follicles. The liver was enlarged and on microscopic examination disclosed the presence of numerous lymphomatous foci in the perilobular connective tissue. The kidneys were enormous, and the interstitial tissues were richly infiltrated by lymphoid

cells. The cervical, inguinal and retroperitoneal lymph nodes were hyperplastic. The gastrointestinal tract presented no noteworthy changes. It is now believed that Cohnheim's original case of pseudoleukemia was in reality an example of that variety of lymphoid leukemia in which the white cells are reduced in number and among which there are abnormal forms—the so-called aleukemic leukemia or aleukemic myelosis. In 1838 Briquet described and illustrated a remarkable disease which has since received the dubious designation of gastrointestinal pseudoleukemia. It seems to be a clinical and pathologic entity. Although it lacks the infiltrative and destructive qualities of a cancerous growth, it is nevertheless regarded by some as a form of lymphosarcoma. Thus far only 15 cases appear to have been recorded, but the disease is probably commoner than the number of published cases indicates, for 2 patients with well marked forms of it and a third in whom its proportions were comparatively limited have been examined post mortem at Bellevue Hospital (Symmers). Anatomically, the disease is marked by an almost incredible degree of lymphoid hyperplasia extending from the base of the tongue, through the esophagus, the stomach and intestine, including the appendix, to the anus, oftenest in the form of closely packed nodules, most numerous in the stomach and intestine, varying in diameter from 1 to 5 mm. but sometimes projecting as polypoid masses measuring several centimeters in height. Peyer's patches are often hyperplastic to an extreme degree, occasionally ulcerated. The spleen is practically always greatly enlarged, as are the lymph nodes of the abdomen and the chest. The superficial nodes in various localities are apt to participate to an almost equally remarkable extent.

Banti's Disease.—Banti's disease, according to the original description of the Italian pathologist, is one of several forms of "splenic anemia" and is divisible into three symptomatic stages—the preascitic, the intermediary and the ascitic. The preascitic stage is initiated by insidious hyperplasia of the spleen and by anemia of the secondary type with leukopenia. At this stage the liver is smooth or only slightly irregular, the sclerotic changes in it being so limited as not to interfere with the portal circulation. The intermediary stage is attended by digestive disturbances, dependent on chronic passive congestion of the gastrointestinal mucous membrane, and occasionally by hemorrhages due to rupture of esophageal varices. In the ascitic stage, anemia is extreme, the patient is emaciated, the abdomen fills with serous fluid and death supervenes. In these circumstances necropsy reveals cirrhosis of the liver together with enlargement of the spleen, the organ often reaching into the pelvis, and weighing 1,200 Gm. or more. Osler defined Banti's disease as "an intoxication of unknown nature characterized by great chronicity. There is a primary progressive enlargement of the spleen

which cannot be correlated with any known cause, anemia of the secondary type with leucopenia—a marked tendency to hemorrhage from the lower oesophagus and a terminal state with cirrhosis of the liver."

There are at least two views concerning the nature of so-called Banti's disease. According to Norris, Symmers and Shapiro [33] (1917), it is a lesion due to syphilis, the changes in the spleen being those of chronic interstitial splenitis, while the changes in the liver may be those of cirrhosis of the atrophic or Laennec type or those of hepar lobatum. In a syphilitic spleen Marchand found histologic changes identical with those described by Banti and advanced the view that many cases of so-called Banti's disease are instances of the preservation of congenital syphilitic splenomegaly. Similar cases have been reported by Copeland, Giffin [26] and others. One of Giffin's 3 patients with this disease was a woman aged 40 who stated that she had been aware of enlargement of the spleen for twelve years. At operation it was observed that the liver was smooth. The spleen was removed; it measured 30 by 17 cm. and weighed 900 Gm. Microscopic examination showed diffuse fibrosis corresponding to that described by Banti, and there were numerous spirochetes in the walls of the splenic vessels.

According to another view, so-called Banti's disease results from a variety of primary lesions which produce hypertension in the splenic vein, followed by congestive splenomegaly and cirrhosis of the liver (Thompson,[34] 1940; Karsner,[35] 1938; Foot,[36] 1945).

Splenectomy is often advised in cases of Banti's syndrome on the ground that removal of the spleen results in improvement or disappearance of anemia. It is also held by some that splenectomy is followed by cessation or prevention of cirrhotic changes in the liver. It is difficult to understand how one arrives at the latter conclusion without the gift of prescience, which presupposes knowledge of events before they happen.

Agnogenic Myeloid Metaplasia of the Spleen.—Under the title "The Human Spleen as a Hematoplastic Organ," Donhauser in 1908 recorded the case of a man aged 58 who presented during life the signs of a greatly enlarged spleen attended by secondary anemia. At necropsy, in addition to replacement of the spleen by the changes of myeloid metaplasia, "the bone marrow removed from the middle portion of the right femur" was found on microscopic examination to present "enormous hyperplasia of the cellular and connective tissue elements." Except for the fact that neither abnormal white cells nor nucleated red cells were

33. Norris, C.; Symmers, D., and Shapiro, L.: Am. J. M. Sc. **154**:893, 1917.

34. Thompson, W. P.: Ann. Int. Med. **14**:255, 1940.

35. Karsner, H. T.: Human Pathology, ed. 6, Philadelphia, J. B. Lippincott Company, 1938, p. 422.

36. Foot, N. C.: Pathology in Surgery, Philadelphia, J. B. Lippincott Company, 1945, p. 170.

present in the blood stream, Donhauser's case was a replica of the disease which has since been described by the Italians as "erythremic myelosis" or "erythremia" and in this country as "agnogenic myeloid metaplasia of the spleen." Clinically, the disease is attended by weakness, pains of the joints, epistaxis and loss of weight, occasionally by jaundice, by slowly progressive enlargement of the spleen and by the presence in the blood stream of immature red and white cells. The consensus is that agnogenic myeloid metaplasia of the spleen is an entity. There are two views concerning its nature. According to one view, it is a variant of myeloid leukemia. However, there are those who believe that nucleated red cells occur in the blood stream of myeloid leukemia only in comparatively rare instances—that they are not constantly present. Except in those cases of myeloid leukemia in which immature red cells originate in extramedullary foci of hemopoiesis, they are not an integrant of the leukemic process but of the superimposed secondary anemia. In agnogenic myeloid metaplasia of the spleen, it is generally believed nucleated red cells are constant, both in the spleen and in the blood stream, irrespective of secondary anemia. Because of this difference some pathologists hold the view that myeloid leukemia and agnogenic myeloid metaplasia of the spleen are independent diseases. Still others point out that were it not for the nucleated red cells present in the spleen the changes might easily be mistaken histologically for those of Hodgkin's disease, which, according to both Symmers and Medlar, is a process of myeloidization of the spleen and of other lymphoid structures following colonization of cells derived from the bone marrow, notably megakaryocytes. In fact, the splenic changes in agnogenic myeloid metaplasia have several times been called "atypical" Hodgkin's disease. In other cases the disease has been included in the indeterminate category of so-called splenic anemia and, because the patients may be jaundiced, it has been mistaken for acquired hemolytic icterus. In that form of splenic anemia known as Mediterranean or Cooley's microcytic anemia, the spleen is often greatly enlarged and may show myeloid metaplasia of the same type as that which occurs in agnogenic myeloid metaplasia.

A disease similar to, if not identical with, agnogenic myeloid metaplasia of the spleen has been described by Ballim and Morse under the heading of myelophthisic splenomegaly. They expressed the opinion that it is "a new clinical and pathologic type of splenomegaly." This opinion is based on the examination of 2 enlarged spleens that had been surgically removed. One was from a woman aged 34; the other, from a man aged 43. Microscopically, both spleens were slightly fibrosed; nucleated red cells were present in moderate abundance together with a few polynuclear giant cells of the megakaryocytic type. Although histologic investigation of the bone marrow was not done, and roentgenologic examination of the

bones revealed no abnormality, these authors nevertheless maintained that "the most likely explanation . . . is that . . . the bone marrow has failed . . . as a depot for the formation of blood cells and that extra marrow islands of hematopoiesis had been developed to compensate for marrow deficiency." On the other hand, according to Jackson, Parker and Lemon, histologic examination of the bone marrow may show that it is "normal, fibrotic, fatty or hyperplastic" and that "it is by no means certain that bone marrow failure is the primary lesion."

Fourteen cases of agnogenic myeloid metaplasia of the spleen have been recorded by Jackson, Parker and Lemon and by Reich and Rumsey. Of this number, splenectomy was done in 7 without apparent signs of improvement. In 3 of the 14 cases irradiation was carried out, but the results were no less disappointing than those observed in the patients who had been subjected to splenectomy. It appears that in agnogenic myeloid metaplasia of the spleen removal of the myeloid foci by splenectomy or depression by irradiation are not only useless but may be harmful. According to Reich and Rumsey, "Splenectomy and irradiation are definitely contraindicated."

NEOPLASMIC SPLENOMEGALIES AND CYSTS

Of the primary noncancerous tumors of the spleen, cavernous hemangioma is perhaps the commonest. Others are fibroma and chondroma. Among primary cancers of the spleen are spindle cell sarcoma and lymphosarcoma. Both are rare. In a case of the former submitted to postmortem examination at Bellevue Hospital, the spleen weighed 1,130 Gm., and metastatic deposits were present in the lymph nodes, the liver, the bone marrow, the kidneys and the subcutaneous tissues. The incidence of primary or preponderant lymphosarcoma of the spleen is indicated by the fact that it was encountered only once in a combined total of 100 cases of lymphosarcomatosis recorded by Kundrat, Ghon and Roman, MacCallum and Symmers.

Kaposi's "multiple idiopathic, hemorrhagic and pigmented sarcoma" manifests itself most often in the skin, although changes may be present in the deeper parts, notably in the gastrointestinal tract and sometimes in the spleen, the liver, the lymph nodes and other organs. Kaposi's disease is remarkable, among other things, for its long duration. Its average span is estimated to be between eight and ten years, but in a number of instances it has been known to persist for periods of from twenty-five to forty-one years. By far the larger number of patients encountered are male Jews and Italians over 40 years of age. It is rare in full-blooded Negroes and is practically unknown in mulattoes and Mongolians. Relatively few cases seem to have been investigated post mortem, Symmers [23e] (1941) having been able to find reports of only 9 necropsies, to which he added 1 of his own. In Symmers' case the

lesions occurred most frequently in the skin and the gastrointestinal tract but also appeared in the tracheobronchial lymph nodes, in the lower lobe of the right lung, in the liver and in the spleen. The spleen extended 7 cm. below the costal margin on the left side and weighed 1,000 Gm. The organ was practically completely replaced by circumscribed tumor nodules, which presented the same histologic features as the growths occurring in the skin and elsewhere.

Under the title of reticuloendotheliomatosis Symmers and Hutcheson[37] (1939) have described a disease that involved not only the organs of the reticuloendothelial system—spleen, lymph nodes, bone marrow and liver—but also structures which are not customarily included in that system, among them the pericardium and the pleura, both adrenal glands, the pancreas, the small intestine, the gallbladder, the kidneys, the uterus, the Fallopian tubes and ovaries and the thyroid gland.

The individual tumors were characterized histologically by overgrowth of minute channels that were lined by argyrophilic endothelial cells provided with spinelike cytoplasmic processes. The spleen was greatly enlarged, as were the lymph nodes. The marrow was extensively replaced by tumor growth, and the patient presented the picture of a variety of myelophthisic anemia. The patient was a white woman aged 42. She complained that for eight months she had suffered from constant generalized "drilling" pains referable to the bony system, together with a tender mass in the upper part of the abdomen on the left side. The skin presented a diffuse lemon yellow tint. Petechiae and small ecchymotic extravasations were visible in the skin of both arms and legs. The cervical, axillary and inguinal nodes were palpably enlarged. The spleen was felt at the crest of the left ilium and was tender. The edge of the liver extended 6 cm. below the level of the costal slope in the right mamillary line, and the organ was likewise tender. Palpation elicited tenderness throughout the bony system.

Blood counts made over a period of several months averaged 1,500,000 red cells. The hemoglobin content varied from 25 to 30 per cent. The white cells numbered on the average 20,800 per cubic millimeter, and there was a high percentage of polymorphonuclear neutrophils, together with many normoblasts, megaloblasts and macrocytes, a few reticulocytes and an occasional myelocyte, and anisocytosis. The color index varied from 1 to 1.13. The platelet count averaged 14,430; the coagulation time, one and a half minutes; the bleeding time, five and a half minutes. The icteric index was 5.

At necropsy there was marked edema of the lower extremities, the left hand and the region of the right breast. The peritoneum contained about 2,000 cc. of thin straw-colored fluid. Embedded in the pericardium were numbers of sharply demarcated grayish nodules, from 1 to 3 mm. in diameter. The pleura of both lungs was studded with smooth opaque grayish areas of thickening together with numerous nodules of the same size and character as those encountered in the pericardium. The spleen weighed 1,150 Gm. The organ was red and tough, the capsule smooth. On section the substance was riddled by ill defined opaque grayish foci measuring from 3 to 6 mm. in diameter. The liver weighed 3,075 Gm. Scattered through the capsule were moderate numbers of dome-shaped grayish nodules varying from 2 to 15 mm. in diameter. Strewn through the substance

37. Symmers, D., and Hutcheson, W.: Arch. Path. **27**:562, 1939.

of the organ were nodules of the same character. The walls of the gallbladder contained a few nodules of similar appearance, averaging about 6 mm. in diameter. In the pancreas were moderate numbers of nodules of much the same size and appearance. The adrenal glands were greatly increased in size and riddled with poorly defined grayish nodules varying in size from 1 to 4 cm. Scattered through both kidneys were a few nodules measuring about 2 mm. in diameter, differing in no essential from those encountered elsewhere. Embedded in each ovary was a solitary nodule, 1 cm. in diameter. The walls of the fallopian tubes were thickly strewn with nodules, each of which was about 2 mm. in diameter. Two subserous sessile nodules measuring, respectively, 3 and 8 mm. in diameter, were present in the fundus of the uterus and several nodules about 4 mm. in diameter were buried in the endometrium. The cervical, peribronchial, anterior mediastinal, mesenteric and pelvic lymph nodes were enlarged, varying in diameter from 1 to 3 cm. The retroperitoneal nodes were extensively involved, and those in the celiac region formed a mass 10 cm. in diameter. The marrow of the pubic bones, lumbar vertebrae and ribs was practically completely replaced by firm, opaque grayish tissue. In the upper part of the right lobe of the thyroid gland were two grayish nodules which measured about 1 cm. in diameter.

Histologically, the lymph nodes were partially or completely replaced by an overgrowth of capillary channels limited externally by threadlike membranes, lining which were continuous or broken layers of irregularly shaped cells containing scanty, slightly acidophilic cytoplasm with spinelike processes. The cytoplasmic processes were sometimes to be seen in sections stained with hematoxylin and eosin but were best shown when impregnated with silver. The structure of the spleen was almost completely obscured by an overgrowth of pinkish staining, poorly nucleated tissue which divided the substance of the organ into islands of different shapes and sizes and of variable cell content. In sections impregnated with silver the supporting tissue was found to consist of a dense network of reticulum fibers. In places it surrounded islands of pulp and sent individual reticulum fibers into the pulp. In other places were islands made up of capillary vessels lined by argyrophilic endothelium with spinelike processes. In the bone marrow many of the interstices were filled with capillary channels lined by endothelial cells with argyrophilic cytoplasmic processes. In silver preparations the intercanalicular tissue was shown to consist of interlacing reticulum fibers. The lobular structure of the liver was lost, as was the columnar arrangement of the parenchymal cells. The sinusoids were widened; Kupffer's cells were unchanged. In the perilobular connective tissues were numerous collections of capillary vessels lined by endothelial cells of a description identical with that of the capillaries observed in the lymph nodes, the spleen and the bone marrow. The histologic changes in the pericardium, the adrenal glands, the kidneys, the small intestine, the pancreas and the uterus, including the myometrium and the endometrium, were practically the same as those in the lymph nodes, the spleen, the bone marrow and the liver.

In short, the disease reticuloendotheliomatosis is characterized not only by myelophthisic anemia but by multiple small nodules in various organs, including the pericardium and the pleura, both adrenal glands, the pancreas, the small intestine, the gallbladder, both kidneys, the uterus, the fallopian tubes and ovaries and the thyroid gland, as well as the reticuloendothelial apparatus, the unit of growth consisting of minute channels lined by argyrophilic endothelial cells with spinelike processes and an overgrowth of reticulum fibers, especially in the spleen.

Metastatic Tumors.—In 298 cases in which cancer was encountered, among 5,155 necropsies, at Bellevue Hospital, the splenic pulp contained metastases 16 times, i.e., in 5.3 per cent of the cases. In most instances the metastatic deposits were small in size and number, seldom growing beyond 1 cm. in diameter and rarely exceeding a half dozen. An exception to this is to be found in cases of cancerous melanoma, in which numerous and extensive metastatic deposits are present in the spleen in a considerable percentage of cases.

Cysts.—Traumatism of the spleen is sometimes followed by hematoma, which in turn undergoes liquefaction and encapsulation, forming the so-called traumatic cyst. Parasitic cysts due to encapsulated larvae of Taenia echinococcus (Echinococcus granulosus) occur in the spleen, but are rare.

SPLENECTOMY

From the standpoint of surgical intervention and removal of the spleen, cases of splenomegaly are divisible into two groups. In one group splenectomy is alleviative or curative. Included in this group are those cases of so-called primary tuberculosis of the spleen in which splenectomy is often followed by remarkably favorable results, even if other organs are similarly involved, especially the liver. In the latter event splenectomy removes a seed bed from which the liver derives fresh infections. In so-called primary tuberculosis of the spleen "death invariably follows if the spleen is not removed." In occasional cases of chronic splenomegaly due to syphilis splenectomy may be considered, especially when anemia threatens life and the patient is intolerant of antisyphilitic treatment. In cases of congenital hemolytic icterus and in cases of idiopathic thrombopenic purpura splenectomy is almost always followed by improvement or cure, even in those cases of the latter disease in which thrombopenia is due to the "hypersplenism" of Gaucher's disease. In cases of so-called Banti's disease splenectomy is often advised. In those cases of chronic malarial splenomegaly, or ague cake, in which rupture occurs spontaneously or after trauma, splenectomy is to be considered if signs of hemorrhage indicate that life is in jeopardy. In a second and larger group of cases splenectomy is contraindicated, including: Vaquez-Osler disease (polycythemia vera); the various metabolic splenomegalies, the splenomegaly of Gaucher's disease, occasionally excepted; leukemia; Hodgkin's disease; agnogenic myeloid metaplasia of the spleen, and several others.

Notes and News

Notice.—Requests from libraries, medical societies and individual physicians in various devastated countries are being received almost daily. If physicians have extra copies of medical journals or copies for which they have no further use, it will be greatly appreciated if they will send them to the headquarters of the Association for distribution abroad.

Appointments, Etc.—Robert E. Stowell, formerly assistant professor of pathology in Washington University, St. Louis, has been appointed professor of pathology and oncology at the University of Kansas.

Esmond R. Long, executive secretary of the medical section of the National Tuberculosis Association, has been appointed editor of the *American Review of Tuberculosis.*

A. F. Rasmussen Jr. has been appointed associate professor in the department of microbiology at the University of Wisconsin.

Charles L. Spurr, assistant professor of medicine in the University of Chicago, has accepted appointment as chief of medicine and director of research at the Anderson Hospital for Cancer Research at the University of Texas, Houston.

O. Felsenfeld, formerly of the Mount Sinai Medical Research Foundation, has been appointed director of bacteriology at Cook County Hospital, Chicago.

W. C. Hueper, formerly assistant director and principal pathologist of the Warner Institute for Therapeutic Research, New York, has been appointed chief of the newly created environmental cancer section of the cancer control branch, National Cancer Institute, Bethesda, Md.

Hahnemann Medical College, Philadelphia, has announced the appointment of John E. Gregory professor of pathology and head of the division of pathology and that of Amedeo Bondi, Jr., professor of bacteriology and head of the department.

John R. Heller, former head of the venereal disease division of the United States Public Health Service, has been appointed director of the National Cancer Institute of Bethesda, Md. Dr. Heller will supervise the federal cancer program, including research conducted at Bethesda laboratories, research grants to other laboratories and hospitals, and federal aid in teaching and cancer control work.

Society News.—The twenty-seventh annual meeting of the American Society of Clinical Pathologists will be held in Chicago October 10 to 16 at the Drake Hotel.

An International Congress on Poliomyelitis will be held, July 12 to 17, at the Waldorf-Astoria Hotel, New York.

The centennial celebration of the American Association for the Advancement of Science will be held in Washington, D. C., September 13 to 17.

The American Society for the Study of Sterility will meet at the Congress Hotel, Chicago, June 21 to 22. Information may be obtained from the secretary, John O. Haman, 490 Post Street, San Francisco 2.

The International Society of Pathologists, an association of societies devoted to clinical pathology, was established in Paris, France, Nov. 21 and 22, 1947, in the course of the first International Congress of the Société Francaise de Biologie Clinique. Delegates and observers attended from Belgium, Czechoslovakia, Denmark, France, Great Britain, Greece, Italy, Peru and the United States. A provisional constitution drawn in conformance with the rules of UNESCO for its associated international bodies establishes the governing body of the society as a council or commission of the delegates from the national member societies. The council acts through an executive committee of six members. The English and French texts of the statutes will come up for ratification next summer. Officers were elected as follows: Sidney C. Dyke, Wolverhampton, England, president; D. Durupt, France, and Josef Ungar, Czechoslovakia and England, secretaries; Raoul Kourilski, France, vice president, and W. H. McMenemey, England, treasurer. The second office of vice president was left vacant in the hope that it might be filled by a representative of the United States. The conference defined the term "clinical pathology" as consisting in "the application of pathology and all its related sciences to clinical medicine." National societies devoted to clinical biology as defined are invited to communicate with the secretary-general, D. Durupt, 20 Rue de la Pompe, Paris, or the assistant secretary, J. Ungar, Claxo Laboratories, Greenford, Middlesex, England.

Research in Aviation Medicine.—The Air University School of Aviation Medicine, Randolph Field, Texas, is in receipt of an announcement from Headquarters, United States Air Force, that the school has been allotted one of the limited number of special research positions recently authorized by Congress to attract outstanding scientists to service laboratories. This action of the Air Force is the first of a series designated to create an Air Force Aeromedical Center capable of fulfilling the needs of that force, which emerged from the war as the nation's first line of defense. It is the intention of the School of Aviation Medicine to utilize the new allocation, which carries with it a salary of $10,000 to $15,000 per annum, to establish a civilian position-vacancy for a director of research. Inquiries concerning this vacancy should be addressed to the commandant.

Markle Scholars in Medical Science.—Sixteen young scientists have been appointed as the first group of scholars in medical science under the plan of the John and Mary R. Markle Foundation to support qualified young scientists who wish to make a career in academic medicine. The scholars were selected from candidates nominated by accredited medical schools in the United States and Canada by regional committees appointed by the Foundation. Toward the support of the scholars and their research, the Foundation has allocated a total of $400,000 to their respective medical schools, each school to receive $25,000 payable at the rate of $5,000 annually for five years.

As faculty members of the participating medical schools, the scholars will devote the next five years to teaching and research, at the end of which time they will have had an opportunity to become established teachers and investigators. According to John M. Russell, executive director of the Foundation, an undetermined number of scholars will be appointed each year for the next few years for the long range purpose of strengthening the faculties of medical schools and medical education generally.

Research on Human Reproduction.—The committee on this research of the National Research Council will consider support of biologic, clinical, economic, medical, psychologic and sociologic research dealing with the field of human reproduction in general and with specific problems, including maternal and fetal physiology, the factors controlling conception, the physiology of fertilization and conception, and sterility. For the year 1948-1949, the committee will place emphasis specifically on investigations of the factors controlling conception, fertility and sterility, but other studies will be supported if projects of special significance are presented. Communications regarding grants should be addressed to: Committee on Human Reproduction, National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D. C.

Toxicologic Division.—The University of Buffalo School of Medicine has established a division of toxicology which will be supported by the university and Erie County. The director of the division is N. C. Klendshir, associate in pathology, who will work under K. Terplan, the head of the department.

Awards.—D. B. Phemister, chairman and professor of the department of surgery, retired, University of Chicago, was recently awarded the honorary fellowship of the Royal College of Surgeons of England for outstanding contributions to the pathology of surgical shock and lesions of the bones and joints.

The American Cancer Society, Inc., has awarded $18,935 to the department of pathology of the University of Illinois College of Medicine in aid of an investigation of the ways in which nucleic acids and amino acids relate to cellular growth. I Gersh, associate professor of pathology, will serve as the principal investigator.

The Richard P. Strong Medal for 1947 has been presented by the American Foundation for Tropical Medicine to Neil P. Macphail, the medical superintendent o the east coast division of the United Fruit Company, Quirigua, Guatemala.

The University of Pennsylvania has awarded the degree of D.Sc. to Esmond R. Long, the director of the Henry Phipps Institute, Philadelphia.

Diagnosis of Cancer.—A course in the cytologic diagnosis of cancer based on the smear technic will be given by the University of California from June 21 through July 2, 1948, under the direction of H. F. Trout, professor of obstetrics and gynecology in the medical school of that university. Requests for information should be sent to Dr. S. R. Mettier, The Medical Center, San Francisco 22.

Scholarship in Oral Pathology.—The New York Institute of Clinical Oral Pathology has established an annual scholarship of $1,000 for clinical research in its field. The executive secretary is G. Roistacher, 101 East Seventy-Ninth Street, New York 21.

CORRECTIONS

In the paper by Drs. B. Gerstl, M. Tager and N. A. Marinaro entitled "Pathogenicity of Bagasse" (ARCH. PATH. **44**:343, 1947) a mistake was inadvertently made in quoting the article of Le Mone, Scott, Moore and Koven (*Radiology* **49**:556, 1947) which at that time was unpublished. The passage should read "Le Mone, Scott, Moore and Koven,[5] in a recent report pointing to the low silica content of bagasse ash, considered it improbable that the minute quantity of silica was an etiologic factor in the development of bagassosis."

In the article by Dr. Jonathan Cohen entitled "Normal Variation of the Costochondral Junction," in the February issue (ARCH. PATH. **45**:246, 1948), figures 3 and 4 (pages 255 and 256) were transposed. The legends are correctly numbered.

Books Received

THE PATHOLOGY OF NUTRITIONAL DISEASE: PHYSIOLOGICAL AND MORPHOLOGICAL CHANGES WHICH RESULT FROM DEFICIENCIES OF THE ESSENTIAL ELEMENTS, AMINO ACIDS, VITAMINS AND FATTY ACIDS. By Richard H. Follis Jr., M.D., formerly associate professor of pathology, Duke University School of Medicine, Durham, N. C., now associate professor of pathology, Johns Hopkins Medical School, Baltimore. Pp. 291, with 71 illustrations. Price $6.75. Springfield, Ill.: Charles C Thomas, Publisher, 1947.

The writing of a book for the purpose of presenting the physiologic and morphologic changes which result from deficiences of the essential elements, amino acids, vitamins and fatty acids is a formidable task and apparently would call for one or more sizable volumes. The statements that Follis' book has a total of 291 pages, including bibliography and index, and that only 232 pages are actually devoted to the text might easily be regarded as insinuating that the work is the product of unsuccessful audacity and that another medical "pot-boiler" has been launched. The fact is that Follis has been amazingly successful and has produced a book of unusual merit at an opportune time.

The book is divided into six parts. Morphologic changes receive greatest emphasis throughout the book.

Part I is a consideration of "Dietary Deficiencies in General." While consisting of 11 pages only, it includes an introduction outlining the development of knowledge of deficiency states and their importance in diverse biologic fields. There are also short chapters on "Specific and Non-specific Tissue Changes," "Endogenous Nutritional Deficiencies" and "The Pathogenesis of Nutritional Disease," all written with great lucidity and pertinency and adequately documented by references taken from many fields—pathology, embryology, microbiology, biochemistry and clinical medicine. This part can be reread with profit after a first reading of the book as a whole.

Part II—"The Essential Elements"—presents, in 49 pages, a wholly admirable condensed, yet surprisingly complete, survey of this field. The functions of the essential elements as structural components of tissues in acid-base equilibrium, as integral parts of enzymes and as activators of enzymatic reactions are presented in a short chapter, well documented by references to the literature and illustrated by examples. Brief general considerations are given to "Isotopes," "Ubiquitous Elements of Unknown Function," "Inter-relations of the Elements" and "The Essential Elements and the Periodic Table." Following the general considerations there are chapters on calcium, magnesium, potassium, sodium, sulfur, phosphorus. chlorine, iron, copper, cobalt, manganese, zinc, iodine and fluorine. Each element is considered under the following headings: "Historical," "Biochemical Relationships," "Pathological Effects" and "Summary." The concluding paragraph of each of these discussions concerns the deficiency of the element in man. The pathologic effects receive detailed attention and are well illustrated.

Part III—"The Essential Amino Acids" (12 pages)—is introduced by a chapter on "Proteins in General" and a table summarizing the effects of deficiencies of the ten essential amino acids—tryptophane, lysine, histidine, arginine, phenylalanine, leucine, isoleucine, threonine, methionine, valine—in the various mammalian species studied thus far. The order of treatment of each amino acid is the same as for

the essential elements—"Historical," Biochemical Relationships," "Pathological Effects," "Summary," and an account of the particular amino acid deficiency in man as far as is known.

Part IV—"The Fat and Water-Soluble Vitamins" (116 pages)—naturally forms the bulk of the book. There is a good, but short, introduction. The same plan of presentation used for parts II and III is followed for each of the chapters—"Vitamin A," "Vitamins D," "Vitamins E (alphatocopherol and its homologs)," "Vitamin K," "Ascorbic Acid," "Thiamine," "Riboflavin," "Nicotinic Acid," "Pantothemic Acid," "Pyridoxine," "Choline," "Biotin," "Folic Acid (L. Casei Factor)," "Inositol" and "Para-aminobenzoic Acid." The only criticisms suggested by the reading of this part are that accounts of the effects of excessive administration of certain vitamins, notably A and the D's, would add to the value of the book, that the treatments of rickets and scurvy are a bit overweighted, in consideration of the size of the book, and that some minor details of the pathology of the two conditions are open to other interpretations than those of the author. The discussion of the biochemical relationships of vitamin D does not include problems raised by the consequences of hypervitaminosis D.

Part V—"The Essential Fatty Acids"—consists of only 2 pages because so little is known about the deficiencies. Linoleic, linolenic and arachidonic acids are all treated together under the familiar headings—"Historical," "Biochemical Relationships," "Pathological Effects" and "Fatty Acid Deficiency in Man."

Part VI—"The Pathologic Anatomy of Specific Tissues: A Recapitulation and Comparison"—consists of 20 pages and is preceded by a lengthy table of "The Specific Tissue Changes Associated with Deficiencies of Elements, Amino Acids, Vitamins and Fatty Acids." In this table are listed the epithelial tissues (skin, gastrointestinal tract, eye and parocular glands, liver, pancreas, adrenal glands, genitourinary tract, respiraotry tract, thyroid gland, parathyroid glands, hypophysis), the mesenchymal tissues (connective tissue, cartilage, bone, teeth), the blood-forming tissues, blood vessels and the coagulating mechanism (red blood cells, white blood cells, platelets, blood vessels, clotting mechanism), the muscle tissues (heart, skeletal muscle, smooth muscle) and the nervous tissues (peripheral nerves, brain). In this table, after each organ or tissue listed, Follis gives the essential element, the amino acid, the vitamin and the fatty acid whose absence has been shown to be responsible for a "deficiency" effect. Following this table, 17 pages are devoted to a recapitulation and comparison of the pathologic anatomy of specific tissues. This part of the book is essentially a summary of the pathologic conditions described in the book but is so presented as to indicate inadequacies of knowledge and to suggest to pathologist and biochemist promising fields for integrative research. It is all too short, too condensed, and perhaps a bit diffident in presentation, but serves to reveal the reasonings of the author and should stimulate the minds of careful readers.

As a feat, the book is remarkable for what it contains in so few pages. It could be the product only of a distinguished investigator of deficiency states. As a textbook for medical students it has every requisite merit, including the two most difficult of achievement—the presentation of what is not known as well as what is known of the field. Investigators of deficiency states will find the book of great use because of its complete coverage, in readible form, of present day knowledge and the carefully culled bibliography of 791 references. Biochemists will enjoy the pathologic portions and pathologists will enjoy the biochemical considerations. Both will profit greatly by reading the book and because of its bibliography. The nutritionist will undoubtedly find the book informative and

suggestive. The physiologist will find that it contains much of interest and value to him. The practitioner of medicine who now prescribes vitamins as indescriminately as he once did potassium iodide should also profit by this book.

The reviewer predicts a large sale of this volume and hopes that subsequent editions will grow in size because its author is so competent to make desirable expansions of the exposition of pertinent biochemistry and to extend the scope of the book into nonmammalian and perhaps even into invertebrate fields. The volume is a notable contribution to biologic literature, both as textbook and as a reference book. If Pulitzer prizes were awarded in this branch of literature, Dr. Follis would be an outstanding candidate.

PATHOLOGY OF TUMOURS. By R. A. Willis, M.D., D.Sc., F.R.C.P., Sir William H. Collins professor of human and comparative pathology, Royal College of Surgeons, London. Cloth. Pp. 992, with 500 illustrations. London, England: Butterworth & Co., Ltd.; St. Louis: C. V. Mosby Company, 1948.

This new book by a well known morphologic oncologist is greeted with enthusiasm as a much needed reference book and textbook. The division into a general introductory part, consisting of 12 chapters on the classification, the nature, the causes, the biologic aspects and the statistics of tumors, and a special part of fifty chapters, each devoted to a specific class of tumors, is conventional, although the contents of the parts are not. All tumors of a given site, both "benign" and "malignant," are usually included in the same chapter. The text is accompanied by 500 illustrations, most of which are photomicrographs of excellent quality, selected from the author's material. There is a tendency to emphasize the unusual rather than the ordinary. For example there are 11 illustrations of Paget's disease. Abstracts of cases from the author's files are used as source data, and there are brief sections on animal tumors, both spontaneous and experimental, in nearly every chapter. All of the common and most of the rare tumors are covered. The book is a revelation of how a vast amount of information can be obtained from a relatively small amount of material carefully studied and interpreted with imagination and good judgment. The book is more than merely descriptive; unsolved problems in need of research are mentioned in connection with many tumors. There is variation in quality of the different chapters, but the bibliographies, except those of some of the earlier chapters, appear uniformly good.

The style is clear and concise. The morphologic descriptions are generally brief, since it is assumed that the reader is not a neophyte. The discussions are often spicy, fearless and admirably critical, clearing away much accumulated verbiage. For example, the chapter on pulmonary tumors ably evaluates the minimal etiologic role of influenza, recognizes the nature of the tumors of the superior pulmonary sulcus and bluntly disposes of much unworthy lore that has accumulated regarding the classification and the genesis of pulmonary carcinoma and bronchial adenoma.

This book contains much material recorded in the literature but included in no other textbook on tumors. It is fully aware of the problems of chemical carcinogens and their possible significance in regard to human cancer. This material is thoroughly integrated into the text of nearly every chapter. The author appears to be on safe ground with respect to the exogenous carcinogens but goes almost beyond the limits of present evidence with respect to endogenous carcinogens. Data are not yet at hand to justify the substitution of "carcinogenic stimuli" for "chronic irritation," although the idea is attractive.

As to problems of terminology and classification, the author has taken a firm stand with which, in general, there can be little cause for disagreement. He

has adopted economical words, such as "prostatomegaly," but avoids others, such as "cancer." He usually substitutes "innocent" for "benign." He minimizes the differences between cancerous and noncancerous tumors, pointing out that the tumors of any location and type form a graded series ranging from the "most benign" to the "highly malignant." In the biologic sense, this is correct, but it will receive little sympathy from the pathologist who derives a considerable share of his livelihood and professional pride as reward for the skill with which he draws the line between the two groups on the basis of morphology interpreted in the light of past experience.

Many unconventional views are expressed by the author. Among them may be mentioned a belief that cancers commonly have a multicentric origin and that their subsequent growth, in the early stages, is by progressive cancerization of the surrounding cells; that melanomas originate from epithelium; that Ewing's endothelial myeloma does not occur as a pathologic entity; that no need exists for the term "mesothelioma" to describe some of the peculiar and characteristic tumors of the serous cavities of the body; that Kaposi's sarcoma may be classified with the lymphoid tumors, and that the term "argentaffin carcinoma" is an adequate substitute for "carcinoid." It is hoped that the author will reconsider some of these views in the light of additional evidence for later editions.

In the prodigious amount of material covered, the few gaps and inconsistencies which exist in this as in most first editions are inconspicuous. Some readers may be disappointed by the omission or minimizing of the importance of aspiration biopsy, the help to be obtained from stained smears in the diagnosis of cancer and the differences between cancerous and noncancerous tumors. Others may disagree with the somewhat abrupt dismissal of the value of tumor grading with respect to the estimation of prognosis. The problem of the additive effect of carcinogenic stimuli, a phenomenon that may assume importance in medicolegal decisions, is barely mentioned. After dismissing embryonal rests as unimportant in etiology, the author subsequently resorts to them to explain the origin of tumors such as intra-alveolar epidermoid carcinoma. The introductory chapters are so excellent in many respects that the reviewer hesitates to make adverse comments. However, in the chapter on the experimental production of tumors, from the text and the references cited, the account given would hardly be recognized as describing work in the same scientific world as that of a recently published monograph. These evidences of scientific provincialism are regrettable.

From the point of view of the teacher called on to recommend books to students, there are included injudicious statements, which fortunately are few. One dislikes having students read on page 144 that most of the work done on the chemistry of the blood and other body fluids and tissues of cancer patients or tumor-bearing animals has been wasted effort. Surely it has not been wasted effort, even if it has produced little of immediate usefulness. And it is hoped that the author goes beyond the bounds of truth when, on the same page, he states that the hope that biochemical studies of the cancerous patient will reveal anything fundamental regarding the nature and the growth of cancer, or will lead to diagnostically useful tests, has so far proved vain and is likely to remain so. Such pessimism is unwarranted.

Finally, it must be stated, with regret, that the volume contains a few errors and misstatements of fact. These vary from minor misspellings, as that of the name of Percivall Pott (page 29), to such misstatements as that mice with spontaneous tumors are usually at least 18 months to 2 years old (page 93), that 3,4-benzpyrene is the most active carcinogen in coal tar (page 34), that squamous metaplasia

rarely occurs in bronchial disease (page 126) and that gastrointestinal cancers have not been experimentally produced (page 38).

The good features of this book far outweigh its defects, and it is highly recommended.

The book reveals an understanding of the morphology and natural history of tumors which is both broad and profound. Some of the ideas are not in accord with current teaching, and convincing evidence for them is not always forthcoming. But the work is critical and tempered with justice. It is a major and stimulating contribution surpassed by no other in its field, oncology, and the author is to be congratulated.

MAN—WEATHER—SUN. By William F. Petersen, M.D. Pp. 447, with 294 illustrations. Price $10. Springfield, Ill.: Charles C Thomas, Publisher, 1948.

Medical scientists who claim that meteoropathologic studies have no importance for the practice of medicine, and physiologists and biochemists whose thinking is bound by Bernard's concept of the milieu interne or Cannon's concept of homeostasis, would do well to ponder this volume.

The author's thesis is that the internal environment is not constant and "that the condition of the normal individual (and the patient) varies continuously in a rhythmic pattern in which all biochemical and biophysical processes participate." He further contends "that in the environmental situation the change in the air mass in which the organism exists is probably of major significance in the sum total of the effectors," "that the effector mechanism primarily involves a tide of oxygen inadequacy followed by a phase of correction or overcorrection," and "that the clinical episode, i.e., the awareness of organic disturbance, is merely the recognition of undue disturbance of normal organic rhythm . . ." In other words, the internal environment is not free from external influences, function and dysfunction are meteorologically conditioned, and man, therefore, is not a free and independent agent. This conditioning begins at conception and continues to the time of death, at which time the organism can no longer adequately meet environmental demands. This thesis is not new—indeed, the author acknowledges inspiration from Hippocrates—but the author's investigations, extending over two decades, have been instrumental in stimulating medical scientists to think more holistically about man.

The text of the monograph is divided into five parts. In part I—"Triplets and Weather"—the results of a battery of biochemical, biophysical and psychologic tests directed at three young men of known genetic background are presented. In addition certain pathophysiologic observations are made. These studies were conducted in day by day fashion during a six week period in June and July 1940. The interindividual correlations and the meteorologic correlations of the day by day reactions are presented in graphic and statistical fashion. In part 2—"Reaction of the Population"—the studies on the triplets are extended to a consideration of the psychopathic admissions, births, deaths, suicides, the sex ratio of the dead, the sex ratio of conception, the twinning and the vital index of June and July 1940. Variations of the "constitution" of the triplets are correlated with these daily reactions of the population of Chicago in graphic and statistical fashion. In part 3—"Man as a Cosmic Resonator"—consideration is given to cycles of longer amplitude in the organic rhythm and in the morbidity and mortality arising from infectious and organic diseases. Weekly and lunar cycles are demonstrated. In part 4—"The Symphony of Sun and Man"—consideration is given to the possible integration of human reactions—epidemics, death, blood p_H, conceptions, weight-length ratio of the newborn, twinning and genius—and solar rhythms of

longer range. In part 5—"The Great Year"—the author discusses the long range trends in cultures and civilizations in relation to solar and climatic cycles and speculates regarding the future of our own civilization. At the end of each part notes on correlated material and references are given. There are 21 tables and 294 figures, as well as subject and author indexes, and an appendix of three tables in which the day to day observations on the triplets are tabulated.

In general, although the subject matter is complex and touches many seemingly unrelated fields, the style of presentation is lucid, and the thesis takes shape as the various data are discussed. The graphs and the statistical studies make the thesis convincing by demonstrating its validity. This reviewer feels that here is an "unfolding of an orderly and rational vista of the human in the framework of the cosmos."

GASTRITIS. By Rudolf Schindler, M.D., clinical professor of internal medicine (gastroenterology), College of Medical Evangelists, Los Angeles; senior member of the attending staff, Los Angeles County Hospital; consultant in gastroenterology, Birmingham General Hospital, Veterans Administration, Van Nuys, Calif.; consultant in gastroenterology, Cedars of Lebanon Hospital, Los Angeles. Pp. 462, with 98 illustrations. Price $10. New York: Grune and Stratton, Inc., 1947.

This book, written by an outstanding student of gastritis, is of interest because of chapters on classification, histopathology and gross pathology, and for attempts to correlate pathologic changes with gastroscopic appearances and clinical symptoms. The tone of the presentation is aptly epitomized by the first two sentences of the preface, which read: "Gastritis is one of the most debated diseases. Its importance is often questioned." The author's conviction of its great importance is not long left in doubt.

Schindler emphasizes the need for unobjectionable material in the study of gastritis. Such material can be obtained only from sites previously selected at gastroscopy in stomachs which contain acid, by biopsy performed without clamps or manipulation. Fourteen biopsy specimens so obtained were added to 41 other specimens. Abstracts of the records of the 55 cases are followed by a section containing 94 excellent photographs and photomicrographs made in connection with the cases. These specimens, records and photographs representing normal stomachs and various types of gastritis, hypertrophy and atrophy, constitute the principal objective material of the book. This material illustrates the author's ideas of classification and nomenclature and the relationships of the types. A bibliography of over 400 items and a thorough index close the book.

In the study of gastritis the major problems have been the incidence, the classification, the clinical significance and the specific symptom complex of each type, and its role as a precancerous condition. This book contributes information on these problems to varying degrees; one may question whether it solves them. Dealing as it does with selected material, it cannot settle the general problem of incidence. It offers a classification which appears to be excellent, at least as a working basis. The problems of the clinical significance and the symptomatic character, especially of the chronic forms of gastritis, need and will, no doubt, receive considerable additional study. The histopathologic classification is clear, but one wonders about transitional, mixed and intermediate types and the convertibility of types. The importance of degenerations in producing atrophic gastritis also remains unsettled.

A study of asymptomatic controls appears desirable for the solution of some of the problems of gastritis and for establishing the limits of the normal. Additional objective, subjective and statistical data of a high order of critical excellence

and supplemental experimental work appear to be indicated. This book represents much experience, thought and effort. Whether one agrees with all the conclusions or not, it nevertheless is stimulating and highly informative, and represents an important milestone in the development of present knowledge and understanding of this complex disease.

THE EPITHELIA OF WOMAN'S REPRODUCTIVE ORGANS: A CORRELATIVE STUDY OF CYCLIC CHANGES. George N. Papanicolaou, M.D., Ph.D., professor of clinical anatomy, Cornell University Medical College; Herbert F. Traut, M.D., professor of obstetrics and gynecology, University of California Medical School, and Andrew A. Marchetti, M.D., associate professor of obstetrics and gynecology, Cornell University Medical College. Pp. 55, with 23 plates (21 colored). Price $10. New York: The Commonwealth Fund, 1948.

This is a 48 page text which contains 22 plates representing photomicrographs and drawings mostly in color.

In the introduction the authors briefly recount the development of knowledge concerning the function and the cyclic anatomic changes of the pelvic organs of generation. The monograph is based on a study of anatomic material from 233 women and innumerable correlated smear preparations. The emphasis throughout is on the cytologic aspects of the epithelium of the female generative tract. The authors do not state in how many instances they have had the complete generative tract, i.e., tubes. ovaries, uterus and vaginal segment, for correlative study. In speaking of early corpora lutea they make no mention of recovering ova from the uterus or the tubes for the purpose of proving whether the ovulation was recent. Chapter 4 is given over to a discussion of the cyclic anatomic and secretory phases of the graafian follicle. On page 11 the ages of the corpora lutea found in their material are described. Their method of determining when ovulation occurred, however, is not indicated. Following the discussion, on pages 16 and 17, of the fat present in the corpus luteum cells and its reaction to various fat stains, a consideration of the general significance of these changes, if they are known, would be interesting and of real help to most readers. The ability to identify the age of regressing corpora lutea raises an old intriguing and contraversial question. In their attempt to answer it the authors submit no critical proof. Chapters 5 to 8 are given over to descriptions of the epitheliums of the fallopian tubes, the endometrium, the cervix and the vagina. Chapters 9 to 11 tell of the changes observed in smears of material taken from the vagina, the cervix and the endometrial cavity in relation to the menstrual cycle. The plates are segregated at the end of the book and are surpassingly beautiful. Throughout the monograph the reviewer is struck by the comparative absence of direct reference to, and critical documentation of, the large material on which the study is based. It is often difficult to know when the discourse represents a description of generally current concepts or when it is based on independent observation. The entire work is an interesting presentation by three well known workers in this field.

TUBERCULOSIS: A DISCUSSION OF PHTHISIOGENESIS, IMMUNOLOGY, PATHOLOGIC PHYSIOLOGY, DIAGNOSIS AND TREATMENT. By Francis Marion Pottenger, M.D., LL.D., emeritus professor of medicine, University of Southern California School of Medicine; medical director, Pottenger Sanatorium and Clinic for Diseases of the Chest, Monrovia, Calif. Pp. 597, illustrated. Price $12. St. Louis: C. V. Mosby Company, 1948.

"The test of scientific truth is patient collection of facts, combined with bold guessing as to the laws binding the facts together." So stated Bertrand Russell,

and Pottenger has utilized this statement as the basis of his book. The author views tuberculosis as mildly infectious and believes that there is great possibility for its eradication. Primary infection serves as vaccination which affords specific protection. Were it not for the fact that it also furnishes a focus from which future metastases may arise, such primary infection would be entirely desirable. In most cases tuberculosis is curable, and the invaded tissues will heal with proper aid at the proper time, according to the author. When Pottenger first began his practice fifty years ago, every phase of this disease was completely surrounded by ignorance and pessimism. In spite of the increased store of knowledge gained during this period, physicians are still in the twilight zone so far as this disease is concerned. Definite knowledge belongs to science, and all else is speculation, philosophy and dogma, which makes the medical position on phthisiogenesis uncomfortable. Pottenger has attempted to eliminate errors which have made physical examination difficult and unsatisfactory and has described his own diagnostic measures. His approach to the disease is largely immunologic and physiologic. Based on Koch's observation that reinoculation is characterized by localization and healing, Pottenger depicts the dual aspect of tuberculosis as a metastasizing-destructive and localizing-healing disease. When the bacilli overcome resistance, spread and cause destruction, they also bring about conditions in the host which are inimical to their own existence. Spontaneous healing will usually occur in the average patient who has an early limited infection if a proper hygienic regimen is followed when first the patient's tuberculosis begins to spread. Exceptions to this course are the patients of unusual susceptibility. The author's optimism concerning this disease is based on epidemiology, the antituberculosis program, knowledge of the susceptibility of the underprivileged, control of the spreader of infection and vaccination.

Pottenger impresses on one that there is a general lack of all-encompassing scientific knowledge in regard to tuberculosis and presents his fifty years of study and experience as interpretation. While such interpretation is necessarily purely personal, Pottenger has handled this difficult and vague subject very well.

A TEXTBOOK OF CLINICAL PATHOLOGY. Edited by Francis P. Parker, M.D., associate professor of pathology, University of Virginia School of Medicine, Charlottesville, Va. Third edition. Pp. 1023, with 273 illustrations. Price $9. Baltimore: Williams & Wilkins Company, 1948.

This edition was in page proof when the editor suddenly died on Nov. 26, 1947. The two previous editions were reviewed favorably in the ARCHIVES. The contributors are I. Davidsohn (blood groups, serologic tests for syphilis); L. W. Diggs (blood cells and marrow, diagnosis and management of hemorrhagic and thromboembolic diseases, cerebrospinal fluid); Emmerich Von Haam (assay of vitamins and hormones, transudates and exudates, skin and mucous membranes, diagnosis of venereal lesions); Roy R. Kracke (disorders of leukocytes); Ralph McBurney (sputum, immunologic tests); Henry E. Meleney (blood parasites, feces); Francis P. Parker (clinical blood chemistry, tests of liver function, gastric and duodenal contents, urine and seminal fluid, tests of renal function); V. P. Sydenstricker (reticulocytes, disorders of erythrocytes). In the appendix are articles on the microscope, standard solutions, atomic weights, diagnosis of rabies, cultural methods, etc. Throughout, the material has been brought up to date. The illustrations have been revised. There seems to be no explanation of plate 5. The number of pages, 554 in the first edition, is now 1,023. One gets the impression that the book continues to be a useful and comprehensive guide in clinical laboratory work.

THE 1947 YEAR BOOK OF PATHOLOGY AND CLINICAL PATHOLOGY. Pathology: Edited by Howard T. Karsner, M.D., professor of pathology, director of the Western Reserve University Institute of Pathology, Cleveland; assistant editor, Herbert Z. Lund, M.D., assistant professor of pathology, Western Reserve University, Cleveland. Clinical Pathology: Edited by Arthur Hawley Sanford, M.D., professor of clinical pathology, University of Minnesota (Mayo Foundation); senior consultant, Division of Clinical Laboratories, Mayo Clinic. Pp. 558, with 103 illustrations. Price $3.75. Chicago: Year Book Publishers, 1948.

The publication of the Year Book of Pathology ceased in 1941. In response to many requests its publication is now resumed, with the addition of a part dealing with clinical pathology. The part on pathology, 312 pages, contains abstracts of important articles on general and special pathology published in 1947 and several concise reviews on special subjects, e.g., cancer and bone pathology, by selected writers. The part on clinical pathology, 224 pages, deals mainly with technical methods published in 1947 in hematology, clinical chemistry, bacteriology, serology, parasitology, clinical microscopy, including gastric analysis and urinalysis. The subject and author indexes cover both parts. The book gives competent summaries of carefully selected recent writings in pathologic fields.

RECENT ADVANCES IN PATHOLOGY. By Geoffrey Hadfield, M.D., F.R.C.P. (Lond.), professor of pathology in the University of London and pathologist to St. Bartholomew's Hospital, and Lawrence P. Garrod, M.D., B.Ch. (Camb.), F.R.C.P. (Lond.), professor of bacteriology in the University of London and bacteriologist to St. Bartholomew's Hospital. Fifth edition. Pp. 363, with 60 illustrations. Price $6.50. Philadelphia and Toronto: The Blakiston Company, 1947.

The previous editions have been reviewed in the ARCHIVES OF PATHOLOGY (**14**:589, 1932; **26**:761, 1938). The present edition has a new chapter on the liver, with an account of recent work on the relationship between hepatic disease and dietary deficiencies and on epidemic hepatitis. The chapter on nephritis contains the appealing simplified classification and study of the evolution of the disease made by Ellis and associates at the London Hospital. Changes have also appeared in the chapters on experimental cancer and on inflammation. It is difficult to take issue with the selection of subjects in a book of this type. Limitations of space and individual preference are the determining factors. Nevertheless, many will miss a reference to the Rh factor. There is occasional evidence of an oversight in revision; for instance, it is stated on page 224 that "twelve years ago there was no clear etiological basis for an anemia not manifestly due to loss of blood, or to destruction of red cells or damage to the marrow by known poisons or infections." The presentation is lucid. Much valuable information has been condensed in the small volume. The illustrations are well selected and reproduced. The references at the end of each chapter have been brought up to date. The subject and author indexes are well organized. The book can be recommended to pathologists and physicians as a good source of reliable and well presented information on recent developments in a few fields of pathology.

AMERICAN MEDICAL RESEARCH PAST AND PRESENT. By Richard H. Shryock, Ph.D., professor of history and lecturer in medical history, University of Pennsylvania; acting director, American Council of Learned Societies. Pp. 350. Price $2.50. New York: The Commonwealth Fund, 1947.

This book is the result of a study made under the auspices of the Committee on Medicine and the Changing Order of the New York Academy of Medicine. As pointed out by John F. Fulton in his preface to the book, "no one previously has attempted to trace the development of research in the medical sciences" and "to any one engaged in medical research, teaching, or medical administration, this much-needed study will prove of challenging interest not only because the story has never been told before, but because it is told so well." The list of the subjects of the chapters gives a good idea of the contents: formative influences; the period of British influence, 1750 to 1820; early support of research, 1860 to 1895; the changing economic and social background; the era of private support, 1895 to 1940; research trends; public relations; public support; the future. At the end of each chapter is a valuable list of notes and references cited in the text. But the book is by no means near to being a complete account of the events by which medical research has grown in this country to its present status. This shortcoming is frankly acknowledged by the author in the following statement: "In this account of American medical research the majority of place references relate to the cities of the northeastern seaboard from Washington to Boston. This emphasis results from two circumstances: first, that in the early period seaboard cities were indeed the main centers of research; and second, that they also afford adequate illustrations of major developments after the Civil War era. However, equally valid references could have been made in the later chapters to the medical centers of the Middle West or, for recent decades, to those of the Pacific coast and of the South." Medical research since 1890 or so in Chicago, for instance, fails to receive due consideration. It may be hoped that such shortcomings will be remedied in future editions.

NEW YORK ACADEMY OF SCIENCES. Special Publications, vol. 4. Editors: Roy Waldo Miner, Myron Gordon and Lothar Salin. The Biology of Melanomas. By Myron Gordon and others. Being the Results of a Conference on the Biology of Normal and Atypical Pigment Cell Growth, Held by the Section of Biology of the New York Academy of Sciences, November 15 and 16, 1946, New York. Pp. 466, with 107 plates. Price $5. New York: New York Academy of Sciences, 1947.

The foreword by Myron Gordon and the introduction by Ross G. Harrison explain the need at this time of conferences like the one organized by the section of biology of the New York Academy of Sciences on normal and atypical pigment cell growth. It appears that George M. Smith, to whom the book is dedicated, then executive director of the National Advisory Cancer Council, was urging the need of a concerted attack on melanoma, "the most malignant form of cancer." This conference brought together a large mass of information on the pigment cell problems of man and other animals, now available in the 27 articles in this book, by different authors. Besides articles on pigmentation in general and articles on the pigment cell problems of man, fishes, the mouse, the axolotl, and birds, there are on the human side articles by Masson on pigment cells in man, by Pack on the clinical study of pigmented nevi and melanoma, by Eleanor Macdonald on malignant melanoma in Connecticut, by Madge T. Macklin on the genetic aspects of pigment cell growths in man, by Helenor C. Wilder on uveal melanoma, by S. William Becker on dermatologic studies of melanin pigmentation, by James B. Hamilton on the influence of the endocrine status on pigmentation in man and other mammals, by Blum on light and the melanin in human skin, and by Greenstein on the chemistry of melanoma. Myron Gordon writes on the role of genes in the melanoma of certain fishes. Other articles deal with the biochemistry and the genetics of pigmentation in mammals, with transplanted mouse melanoma, with

the chemistry of pigmented cells, with the classification of melanins, with the hormonal regulation of feather pigmentation, with color changes in vertebrates, with amelanotic myeloma and with the melanoblast as a mesodermal cell. The book is elaborately illustrated by no less than 107 plates, mostly in black and white. Masson's article, for instance, has 15 plates, Becker's 23, Levine's (typical and amelanotic myeloma) 13 and Gordon's 12, some in color. There is a good index, subject and author combined. As stated in the foreword, the grants-in-aid for publication of this book indicate the interest in the problems of melanoma. The book marks a promising start of effective cooperation in this field of research.

A Text-Book of Pathology: An Introduction to Medicine. By William Boyd, M.D., Dipl., Psych., M.R.C.P. (Edin.), F.R.C.P. (Lond.), LL.D. (Sask.), M.D. (Oslo), F.R.S.C., professor of pathology and bacteriology, University of Toronto, Toronto. Fifth edition, thoroughly revised. Pp. 1049, with 500 illustrations and 30 plates in color. Price, $10. Philadelphia: Lea & Febiger, 1947.

All parts of the book bear evidence of thorough revision. A great deal of new material has been added. There are many new sections and new illustrations. The author has been true to the ideal he mentions in the preface: "that to perfection's sacred might I nearer still may rise." The book continues to be an excellent textbook of general and special pathology. In future editions the methods of staining should be mentioned in connection with all colored illustrations, and "cancer" and "cancerous" used more consistently in place of "malignancy" and "malignant." One reads on page 249 that it is "easy to divide tumors into two great classes: the one innocent, simple or benign, the other malignant. This is true both for epithelial and connective tissue tumors. In the former carcinoma and in the latter sarcoma is the malignant representative. All malignant tumors are included under the common heading of cancer . . ." A closer adjustment to the current rejection of the terms "malignant" and "malignancy" as ambiguous synonyms of "cancerous" and "cancer" seems indicated.

Sexual Behavior in the Human Male. By Alfred C. Kinsey, professor of zoology, Indiana University; Wardell B. Pomeroy and Clyde E. Martin, Research Associates, Indiana University. Preface by Alan Gregg, M.D., director, Division of Medical Sciences, Rockefeller Foundation. Pages 804, with 173 charts and 159 tables. Price $6.50. Philadelphia: W. B. Saunders Company, 1948.

This book is based on surveys made by the authors under the supervision of the Committee on Problems of Sex of the National Research Council by means of funds provided by the medical division of the Rockefeller Foundation. The research has been carried on during the past nine years and includes a study of more than 12,000 persons of different ages and levels, economic and social. There are three parts: history and method; factors affecting sexual outlet; sources of sexual outlet. This extensive scope of facts hitherto not available throws new and much needed light on male sex problems at different social, economic and age levels. The book marks a decisive step "toward basing the knowledge of the sexual impulse on a factual scientific foundation rather than on traditional and speculative thinking."

Histopathologic Technic. By R. D. Lillie, M.D., medical director, United States Public Health Service; chief, National Institute of Health Pathology Laboratory. Pp. 300, with numerous tables. Price $4.75. Philadelphia and Toronto: The Blakiston Company, 1948.

This book is a scholarly evaluation of the various fixatives and of routine and special histologic stains. The author gives consideration not only to older, acceptable and tried methods but also to the recent improvements of histologic

technic. He refers frequently to his own extensive experience. The importance of the histochemical demonstration of enzymes is stressed, and fluorescent microscopy and polariscopy are discussed. References to more detailed studies of certain methods are plentiful. Technicians, students and pathologists will find this new book a handy manual. It is thoroughly readable and provides a number of helpful tables. The wealth of useful information and advice which this book provides makes it a most important addition to the bookshelf of any modern laboratory.

PATHOLOGICAL HISTOLOGY. By Robertson F. Ogilvie, M.D., F.R.C.P. (Edin.), F.R.S.E., lecturer in pathology and assistant in forensic medicine, University of Edinburgh; senior pathologist, Royal Infirmary, Edinburgh; pathologist to the Leith and Deaconess Hospitals, Edinburgh; examiner in pathology and forensic medicine for the Triple Qualification. Foreword by A. Murray Drennan, M.D., F.R.C.P. (Edin.), F.R.S.E., professor of pathology, University of Edinburgh. Third edition. Pp. 459, with 260 photomicrographs in color. Price $10. Baltimore: Williams & Wilkins Company, 1947.

The first edition, 332 pages, was published in 1940.

HARVEY CUSHING: A BIOGRAPHY. By John F. Fulton. Pp. 754, illustrated. Price $5. Springfield, Ill.: Charles C Thomas, Publisher, 1946.

This book has been received with uniformly high favor. It is a Book-of-the-Month Club choice. There is no occasion at this time for a detailed review. The book will be of interest and stimulus to students of medicine of all ages, especially the younger. Note its low cost. Shryock, in his history of "American Medical Research, Past and Present" (London: The Commonwealth Fund, 1947) points out that Fulton's "Harvey Cushing" is one of the biographies which "provide splendid accounts of the recent history and significance of medical research."

FACTS AND FIGURES ABOUT INFANTILE PARALYSIS. Publication no. 59, The National Foundation for Infantile Paralysis. Pp. 31. New York: National Foundation for Infantile Paralysis, 1948.

CORONARY HEART DISEASE. By A. Carlton Ernstene, M.D., chief of the Section on Cardiovascular Disease, Cleveland Clinic, Cleveland. American Lecture Series, Publication no. 18. Pp. 95. Price $2.50. Springfield, Ill.: Charles C Thomas, Publisher, 1948.